National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

## NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

## AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Canada

UNIVERSITY OF ALBERTA

# REGULATION OF THE POST-PARTUM ANESTRUS IN THE SOW

by

FABIO DE RENSIS

©

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

IN

ANIMAL REPRODUCTION

DEPARTMENT OF ANIMAL SCIENCE
EDMONTON, ALBERTA, CANADA
FALL 1993

National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-88090-2

Canada

UNIVERSITY OF ALBERTA

Release Form

NAME OF AUTHOR **DE RENSIS FABIO**

TITLE OF THESIS **NEUROENDOCRINE REGULATION OF POST-PARTUM ANESTRUS IN THE SOW**

DEGREE FOR WHICH THESIS WAS PRESENTED **DOCTOR OF PHILOSOPHY**

YEAR THIS DEGREE GRANTED FALL 1993

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as hereinbefore provided neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

Fabio De R

Fabio De Rensis
V. T. Gulli 1, PARMA
43100, ITALY

DATED Oct. 8, 1993

Signa tibi dicam, tu condita mente tenebo:
Cum tibi sollicito secreti ad flumina undam
Litoreis ingens inventa sub ilicibus sus
Triginta capitum fetus enixa iacebit,
Alba, solo recubans, albi circum ubera nati,
Is locus urbis erit, requies ea certa laborum.

Aeneis, book III, 388-393

Keep in the mind the sign I give to you now:
One day, when you are anxious and alone
At the wave of idden river, you will find
Under the oaks on the shore, a sow, a white one,
Immense, with a new-born litter, thirty young
At the old one's udders: that will be the place
Of the city, the certain rest from labor.

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they here read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **NEUROENDOCRINE REGULATION OF POST-PARTUM ANESTRUS IN THE SOW** submitted by **FABIO DE RENSIS** in partial fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in **ANIMAL REPRODUCTION.**

G.R. Foxcroft (Supervisor)

F.X. Aherne

Frank Aherne

S.J Harvey

Steve Harvey

R.M. Couch

N.C. Rawlings (External examiner)

DATE: Oct. 8, 1993

# ABSTRACT

The neuroendocrine mechanisms mediating the suckling-induced inhibition of LH secretion were studied during lactation in the sow. In Experiment 1, repetitive treatment of lactating sows with naloxone from 39h to 78h post-partum was not able to prevent the inhibition of LH secretion observed in untreated sows, whereas naloxone treatment at d 10 of lactation increased LH secretion ($p<0.05$). Naloxone decreased ($p<0.05$) plasma prolactin (PRL) at d 10 but had no effect during the first 78h after farrowing. FSH secretion was unaffected by naloxone treatment. In Experiment 2, use of different naloxone injection schedules confirmed the conclusion of Experiment 1 that opioidergic regulation of LH and PRL secretion in the sow changes during lactation. In Experiment 3 administration of morphine to sows weaned 6h after farrowing (Zero-Weaned) or to normally lactating sows was used to further study opiodergic regulation of LH and PRL. LH increased ($p<0.001$), while PRL decreased ($p<0.001$), in Zero-Weaned compared to Suckled sows. Although morphine decreased LH secretion in both groups ($p<0.05$) and PRL in the Suckled sows ($p<0.05$), these effects were more evident as lactation progressed. In Experiment 4 sows were challenged at d 25 of lactation with naloxone and then GnRH. LH secretion before and after treatment at d 25 and at d 2 post-partum, and immediately after weaning on d 25, were compared with ovarian activity measured the day after weaning. None of these estimates of LH secretion in lactation appeared useful for predicting subsequent reproductive potential.

The effects of continuous treatment with a long acting dopamine agonist (cabergoline) from d 11 of lactation on the LH and PRL secretion at d 12, 19 and 26 and interactions with opioidergic mechanisms were assessed in Experiment 5. Cabergoline treatment increased ($p<0.05$) mean plasma LH concentrations at d 26 but not at d12 and 16 of lactation, whilst morphine decreased ($p<0.001$) mean LH levels at day 26 of lactation. Dopamine agonist administration is therefore able to increase LH secretion after chronic and not acute treatment. Finally unchanged plasma LH in the presence of low plasma PRL indicate that PRL per se does not directly influence LH secretion during lactation in the sow.

# ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. George R. Foxcroft for his understanding and support through all my years of study and because he has been able to make this a rewarding experience. Grateful thanks are also given to the Faculty of Medicina Veterinaria of Milano and Parma and to Prof. G. Maffeo and C. Tamanini for allowing me to finish these studies. The financial support of Consiglio Nazionale delle Ricerche (Italy, scholarship N.A.T.O n 204.2469, Altri Interventi and Progetto Bilaterale), Natural Science and Engineering Research Council, Canada and the Alberta Pork Producer Development Corporation are gratefully acknowledged. My thanks also go to the staff of the Department of Animal Science and especially to Dorothy Irwin, Dorothy Payne, Gordon Grant and Lynn Wright. A particular thanks to the graduate students and postdoctoral fellows of our group: Jianchi Ding, John Cosgrove, Andrew Pharazyn, Sue Charlton, Emma Clowes, Heather Willis, Sigrid Hofäcker and Eduardo Beltranena for their help in my experiments and for their friendship. I wish also to thank my other friends from the University and from Edmonton with whom I shared many moments and that helped my stay in Edmonton to be an enjoyable time.

## TABLE OF CONTENTS

INTRODUCTION . . . . . . . . . . 1

CHAPTER I: LITERATURE REVIEW . . . . . . . . . . 3

1. OVERALL CONCEPTS OF HYPOTHALAMIC CONTROL OF GnRH SECRETION IN LACTATING AND NON-LACTATING FEMALES . . . . . . . . . . 3
1.1 Introduction . . . . . . . . . . 3
1.2 Gonadotropin releasing hormone (GnRH) . . . . . . . . . . 3
1.3 Hypothalamic concentration of GnRH during lactation . . . . . . . . . . 4
1.4 Pituitary GnRH receptors during lactation . . . . . . . . . . 4
1.5 Effect of exogenous GnRH treatment on gonadotropin secretion during lactation . . . . . . . . . . 5

2. MECHANISMS INVOLVED IN CENTRAL REGULATION OF GONADOTROPIN SECRETION . . . . . . . . . . 6
2.1 Adrenaline and noradrenaline . . . . . . . . . . 6
2.2 Dopamine . . . . . . . . . . 8
2.3 Serotonin . . . . . . . . . . 10
2.4 Endogenous opioid peptides . . . . . . . . . . 10
  2.4.1 The opioids and post-partum anestrus in the domestic animals. . . . . . . . . . . 11
  2.4.2 Peripheral endorphin and GnRH secretion . . . . . . . . . . 13
2.5 Other neurotransmitters . . . . . . . . . . 14
2.6 Interactions between opioids, dopamine and gonadotropin secretion . . 14

3. THE GONADOTROPINS AND MECHANISMS MODULATING THEIR SECRETION . . . . . . . . . . 15
3.1 Hypothalamic control . . . . . . . . . . 15
3.2 Ovarian steroid feed-back . . . . . . . . . . 18

4. GONADOTROPIN SECRETION DURING GESTATION, PARTURITION AND LACTATION . . . . . . . . . . 21

5. PROLACTIN AND THE REGULATION OF PROLACTIN SECRETION . . . . . . . . . . 22
5.1 Origin and identity of prolactin . . . . . . . . . . 22
  5.1.1 Topography of prolacting-secreting cells . . . . . . . . . . 22
  5.1.2 Molecular heterogeneity of PRL . . . . . . . . . . 23
5.2 Spontaneous Prolactin secretion . . . . . . . . . . 23
5.3 Autocrine and Paracrine control . . . . . . . . . . 23
5.4 Hypothalamic Control . . . . . . . . . . 24

5.4.1 Prolactin Inhibitory Factors (PIF) .................... 24
5.4.2 Prolactin Releasing Factors (PRF) .................... 26
5.5 Short loop feed-back .................... 29
5.6 Steroid hormones .................... 29

6. INTERRELATIONSHIP BETWEEN PROLACTIN AND GONADOTROPIN SECRETION .................... 29

7. PROLACTIN INFLUENCE ON THE OVARY .................... 34

8. CONCLUSION .................... 35

9. BIBLIOGRAPHY .................... 42

**CHAPTER 2: EXPERIMENT 1: LH AND PROLACTIN RESPONSES TO NALOXONE VARY WITH STAGE OF LACTATION IN THE SOW** .................... 73

2.1 INTRODUCTION .................... 73
2.2 MATERIALS AND METHODS .................... 73
2.2.1 Animals and blood collection .................... 73
2.2.2 Hormone assays .................... 74
2.2.3 Statistical analysis .................... 75
2.3 RESULTS .................... 75
2.4 DISCUSSION .................... 77
2.5 CONCLUSION .................... 79
2.6 BIBLIOGRAPHY .................... 94

**CHAPTER 3: EXPERIMENT 2: OPIOID ANTAGONISM (NALOXONE) FAILS TO REVERSE THE SUCKLING-INDUCED REGULATION OF LH AND PROLACTIN SECRETION IN EARLY LACTATION IN THE SOW** .................... 98

3.1 INTRODUCTION .................... 98
3.2 MATERIALS AND METHODS .................... 98
3.2.1 Animals and blood collection .................... 98
3.2.2 Hormone assays .................... 99
3.2.3 Statistical analysis .................... 99
3.3 RESULTS .................... 100
3.4 DISCUSSION .................... 101
3.5 CONCLUSION .................... 103
3.6 BIBLIOGRAPHY .................... 134

CHAPTER 4: EXPERIMENT 3: INTERACTION BETWEEN SUCKLING AND MORPHINE ADMINISTRATION IN THE REGULATION OF LH AND PROLACTIN SECRETION IN THE SOW ........ 138

4.1 INTRODUCTION ........ 138
4.2 MATERIALS AND METHODS ........ 139
  4.2.1 Animals and blood collection ........ 139
  4.2.2 Hormone assays ........ 139
  4.2.3 Statistical analysis ........ 139
4.3 RESULTS ........ 140
4.4 DISCUSSION ........ 140
4.5 CONCLUSION ........ 142
4.6 BIBLIOGRAPHY ........ 164

CHAPTER 5: EXPERIMENT 4:RELATIONSHIP BETWEEN GONADOTROPIN SECRETION DURING LACTATION AND ENDOCRINE CHANGES AND FOLLICULAR DEVELOPMENT AFTER WEANING IN THE SOW ........ 169

5.1 INTRODUCTION ........ 169
5.2 MATERIALS AND METHODS ........ 169
  5.2.1 Animals and blood collection ........ 169
  5.2.2 In vitro study ........ 170
  5.2.3 Hormone assays. ........ 170
  5.2.4 Statistical analysis ........ 170
5.3 RESULTS ........ 170
5.4 DISCUSSION ........ 171
5.5 BIBLIOGRAPHY ........ 180

CHAPTER 6: EXPERIMENT 5: CHRONIC ADMINISTRATION OF A LONG ACTING DOPAMINE AGONIST (CABERGOLINE) BLOCKS THE INHIBITORY EFFECT OF OPIOIDS ON LH SECRETION DURING LACTATION IN THE SOW ........ 182

6.1 INTRODUCTION ........ 182
6.2 MATERIALS AND METHODS ........ 183
  6.2.1 Animals and blood collection ........ 183
  6.2.2 Hormone assays ........ 184
  6.2.3 Statistical analysis ........ 184
6.3 RESULTS ........ 185
6.4 DISCUSSION ........ 185

6.5 CONCLUSION . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 188
6.6 BIBLIOGRAPHY . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 205

**CHAPTER 7** . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 212

7.1 DISCUSSION . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 212
7.2 CONCLUSION . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 216
7.3 BIBLIOGRAPHY . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 221

# LIST OF TABLES

**Table 1.** Effect of administration of naloxone on LH secretion in domestic species . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 36

# LIST OF FIGURES

**Fig. 1.1** Possible mechanisms of neurohormonal control of gonadotropin and PRL secretion during lactation . . . . . . . . . . . . . . . . . . . . 40

**Fig. 1.2** Schematic diagram of the suggested interactions between the dopaminergic and opioidergic system and the control of GnRH\LH secretion . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 41

**Fig. 2.1** Mean (+SEM) plasma LH concentrations 66-78h after farrowing in the 3h period before, and the 3h periods after, injection of two doses of naloxone in lactating sows . . . . . . . . . . . . . . . . 81

**Fig. 2.2** Individual plasma LH profiles in four Control sows . . . . . . . . 83

**Fig. 2.3** Individual plasma LH profiles in four Naloxone treated sows . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 85

**Fig 2.4** Mean (+SEM) plasma LH in Naloxone treated (n=6) and Control sows (n=5) . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 87

**Fig 2.5** Mean (+SEM) plasma FSH in Naloxone treated (n=6) and Control sows (n=5) . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 89

**Fig 2.6** Individual plasma prolactin profiles in two Naloxone treated and two Control sows . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 91

**Fig 2.7** Mean (+SEM) plasma prolactin (PRL) in Naloxone treated (n=6) and Control sows (n=5) . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 93

**Fig. 3.1** Schematic diagram of experimental design . . . . . . . . . . . . . . 105

**Fig. 3.2** Individual plasma LH profiles in two sows in group I . . . . . . 107

**Fig. 3.3** Individual plasma LH profiles in two sows in group II . . . . . 109

**Fig. 3.4** Individual plasma LH profiles in two sows in group III . . . . . 111

**Fig. 3.5** Individual plasma LH profiles in two sows in group IV . . . . . 113

**Fig. 3.6** Mean plasma LH concentrations during control pre-treatment periods, and following single and double naloxone (2mg/kg) injections, during four post-partum periods . . . . . . . . . . . . . . . 115

**Fig. 3.7** Mean plasma LH concentrations during control pre-treatment periods, and following treble and quadruple naloxone (2mg/kg) injections, during four post-partum periods . . . . . . . . . . . . . . . 117

**Fig. 3.8** Mean plasma LH concentrations in Control and Naloxone groups for three bleeding periods on days 10 and 11 of lactation . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 119

**Fig. 3.9** Individual plasma PRL profiles in two sows treated with naloxone (group I) . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 121

**Fig. 3.10** Individual plasma PRL profiles in two sows treated with naloxone (group II) . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 123

**Fig. 3.11** Individual plasma PRL profiles in two sows treated with naloxone (group III) . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 125

**Fig. 3.12** Individual plasma PRL profiles in two sows treated with naloxone (group IV) . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 127

**Fig. 3.13** Mean plasma PRL concentrations during control pre-treatment periods, and following single, and double naloxone (2mg/kg) injections, during four post-partum periods . . . . . . . . 129

**Fig. 3.14** Mean plasma PRL concentrations during control pre-treatment periods, and following, treble and quadruple naloxone (2mg/kg) injections, during four post-partum periods . . . . . . . . 131

**Fig. 3.15** Mean plasma PRL concentrations in Control and Naloxone groups for three bleeding periods on days 10 and 11 . . . . . . . . . . . . . . . 133

**Fig. 4.1** Individual plasma LH profile in two Suckled sows treated with morphine . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 145

**Fig. 4.2** Individual plasma LH profile in two Suckled sows treated with morphine . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 147

**Fig. 4.3** Individual plasma LH profile in two Zero-Weaned sows treated with morphine . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 149

**Fig. 4.4** Individual plasma LH profile in two Zero-Weaned sows treated with morphine . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 151

**Fig. 4.5** Mean plasma LH in zero-weaned and suckled sows . . . . . . . 153

**Fig. 4.6** Individual plasma PRL profiles in two Suckled sows . . . . . . . 155

**Fig. 4.7** Individual plasma PRL profiles in two Suckled sows . . . . . . . 157

**Fig. 4.8** Individual plasma PRL profiles in two Zero-Weaned sows . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 159

**Fig. 4.9** Individual plasma PRL profiles in two Zero-Weaned sows . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 161

**Fig. 4.10** Mean plasma PRL in Zero-weaned and Suckled sows . . . . . . 163

**Fig. 5.1** Individual plasma LH profiles in two sows treated with naloxone and GnRH . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 174

**Fig. 5.2** Individual plasma LH profiles in two sows treated with naloxone and GnRH . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 176

**Fig. 5.3** Mean plasma LH in sows treated with naloxone and GnRH . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .177

**Fig. 5.4** Mean follicular volume (ul) for each sow after weaning . . . . 179

**Fig. 6.1** Individual plasma LH profiles in two sows from group CONT and CAB . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 190

**Fig. 6.2** Individual plasma LH profiles in two sows from group CAB+NAL and CAB+MORF . . . . . . . . . . . . . . . . . . . . . . . . . . 192

**Fig. 6.3a** Mean (+SEM) plasma LH in CONT and CAB groups . . . . . . 194

**Fig. 6.3b** Mean (+SEM) plasma LH in CAB+NAL and CAB+MORPH groups . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 196

**Fig. 6.4** Individual plasma prolactin profiles of two sows from CONT group . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 198

**Fig. 6.5** Individual plasma prolactin profiles of two sows from CAB group . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 200

**Fig. 6.6** Individual plasma prolactin profiles of one sow from CAB+NAL and one sow from CAB+MORPH group . . . . . . . . . . . . . . 202

**Fig. 6.7** Mean (+SEM) plasma PRL in CONT, CAB, CAB+NAL and CAB+MORF groups . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 204

**Fig. 7.1** A general scheme summarizing some of the factors that are involved in the control of LH secretion in the sow during lactation . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 218

**Fig. 7.2** Potential exogenous treatments to overcome lactational anestrus and the period of lactation during which they could be effective in overriding the inhibitory effect of suckling . . . . . . . . . . . . . . 220

# INTRODUCTION

The productivity of pig herds is principally based on the number of pigs produced per sow per year and it is therefore dependent on both the annual number of sows farrowing and their litter size at birth. The life cycle of the pig comprises both productive (pregnancy and lactation) and unproductive or "empty-day" periods due to the replacement of sows and the interval from weaning to conception. Due to the fact that the gestation period of the pig is relatively constant, the possibility of improving productivity is based on controlling either lactation length and/or the period from weaning to fertile estrus. Lactation lengths in current commercial practice vary from three to four weeks. Reducing lactation length to less than three weeks often results in a post-weaning "anestrus" defined in practice as the absence of estrus for more than 10 days after weaning.

Many studies have been undertaken with the aim of inducing reproductive activity during lactation by exogenous hormonal treatment. Generally, however, the results were not satisfactory and have not allowed the utilization of these techniques in commercial herds. Therefore, in order to increase sow productivity it is necessary to have a better understanding of the hormonal mechanism(s) that regulate reproductive activity during lactation. It will then hopefully be possible to devise new methods for inducing reproductive activity during this period.

In domestic ruminants there is good evidence that the development of lactational anestrus results from a superimposition of the inhibitory effects of suckling on the latent, suppressive effects of pregnancy (see Nett, 1987). In the sow, however, available information indicates that active gonadotropin and particularly LH, secretion continues into late pregnancy and that despite earlier reports of a lack of LH synthesis and release in the early postpartum period, follicular stimulation can occur immediately after removal of the litter at birth (zero weaning; for review see Varley and Foxcroft, 1989). More recently (De Rensis and Foxcroft, 1993b) it has been demonstrated that active LH secretion occurs in both suckled and zero-weaned sows in the first 48 hours after parturition and only after this time is a significant inhibitory effect of sucking on LH secretion apparent. It has also been observed that in the absence of the suckling stimulus the release of gonadotropins in the immediate post-partum period provides an effective stimulus to the ovary and that ovarian tissue is sensitive to stimulation at this time (De Rensis and Foxcroft, 1993b).

Amongst the mechanisms that regulate reproductive activity after farrowing in the sow, there is evidence for an opioidergic mechanism mediating the inhibitory effects of suckling on gonadotropin secretion in mid and late lactation (Mattioli et al.,1986; Barb et al., 1986); however, such studies have not been extended to the very early post-partum period when the mechanisms blocking follicular development are first activated.

The effects of the dopaminergic system on the control of gonadotropin and prolactin (PRL) secretion during lactation have been extensively studied in the human, rat and sheep (Ben-Jonathan et al., 1989) but essentially no data exist in the sow. Therefore, a better understanding of the hormonal dysfunction that causes reproductive disorders in very early lactating sows will be useful for determining the best therapy for resolving this problem.

The studies described in this thesis were therefore developed in the hope of producing a greater understanding the mechanisms controlling reproductive function in lactating sows and to clarify some of the hormonal disfunctions which occur as a consequence of early weaning. As the experimental work involved a detailed assessment of the endocrine status of the of zero-weaned and early post-partum sow, a review of the neuro-endocrine control of the hypothalamo-hypophysial-ovarian axis is appropriate. This review is presented in the next sections on a comparative basis across species. This forms a basis on which to discuss the reproductive physiology and endocrinology of the lactating sow in later chapters that present the results of the experimental work carried out. Finally, the overall implications of the data obtained are discussed in the context of existing literature in the general discussion.

# CHAPTER 1
# LITERATURE REVIEW

## 1. OVERALL CONCEPTS OF HYPOTHALAMIC CONTROL OF GnRH SECRETION IN LACTATING AND NON-LACTATING FEMALES

### 1.1 Introduction

Initially it was suggested that there were two different releasing hormones for FSH and LH and a putative FSH releasing factor was originally purified by gel filtration on Sephadex G-25 followed by carboxymethyl cellulose (CMC) chromatography by Dhariwal et al. (1967). However since LHRH has intrinsic FSH-releasing activity and since Schally et al. (1971) could not separate FSH- from LH-releasing activity after several chromatographic steps (as monitored by in vitro assays and measurement of FSH by immunoassay) it has become accepted that the synthesis and release of both LH and FSH are stimulated by a single decapeptide hormone (GnRH); nevertheless, numerous studies continue to address this problem. For example Lumpkin et al. (1984, 1989) confirmed the observation of Chappel et al. (1976) that different regions of the hypothalamus may serve as the respective pulse generator for the differential episodic release of LH and FSH and the region of the paraventricular nucleus-dorsal anterior hypothalamic area (PVN-DAHA) has a selective control of tonic FSH secretion. Therefore they suggested that a specific FSH-releasing factor was secreted by the PVN-DAHA. In contrast Urbanski et al. (1987) demonstrated that in the orchidectomized rat the pulsatile pattern of GnRH release was reflected in the pulsatile pattern of not only LH but also FSH secretion. Therefore they concluded that the pulsatile secretion of both gonadotropins was primarily orchestrated by a single hypothalamic releasing hormone. If two separate hypothalamic releasing hormones do indeed exist, it would appear that in the orchidectomized rat their episodic release is tightly coupled to the same hypothalamic pulse generator.

Since a separate FSH-releasing factor has not yet been identified, in the following chapters GnRH will be considered as the main releasing factor for both LH and FSH secretion.

### 1.2 Gonadotropin releasing hormone (GnRH)

The half life of GnRH in plasma has been reported to be about 5 minutes (Nett et al., 1973). GnRH containing neurones do not predominate in clearly defined nuclei but are scattered throughout the rostral and mediobasal hypothalamus (Dyer, 1985). Fluoresin-labelled antibodies have been used to locate areas of high GnRH concentration in the rat (Palkovits et al., 1974, Silverman et al., 1979) and GnRH containing neurones have been detected in the medial preoptic area and in the anterior and arcuate/ventromedial hypothalamus. Other reports on the localization of GnRH containing neurones are conflicting but two distinct areas with a high neuronal content of GnRH have been observed by several groups (for review see Dyer, 1985). This has led to the suggestion that GnRH released from the medial basal hypothalamus may be the major controller of tonic gonadotropin secretion, while the neurones of the preoptic/suprachiasmatic nuclei are involved in the phasic release of LH during the preovulatory gonadotropin surge (Domanski et al., 1980).

One of the biggest problems in measuring GnRH secretion has been to find a method for directly monitoring GnRH levels in a sample of hypophysial portal blood. Different techniques have been studied in recent years. One involves accessing the anterior face of the pituitary gland by unilateral trans-nasal trans-sphenoidal surgery (Clarke and Cummins, 1982); a second technique is the "push-pull" perfusion system which was originally developed by Levine et al. (1982) in the sheep and later adapted in the monkey (Levine et al., 1985). Finally, a technique has been developed that is based on the withdrawal of cerebrospinal fluid from the third ventricle (Van Vugt et al., 1983) in the monkey. Each of these techniques has advantages and disadvantages when studying GnRH release. In the pig and other species therefore, information on the synthesis and release of GnRH has been obtained indirectly by measuring hypothalamic GnRH content and by comparing patterns of gonadotropin secretion. It is generally accepted that there is a direct relationship between LH pulse release from the pituitary and GnRH pulses in hypophyseal portal blood (Clarke et al., 1982; Levine et al., 1982).

### 1.3 Hypothalamic concentration of GnRH during lactation

Crowder et al. (1982) reported that the concentration of hypothalamic GnRH does not change during the post-partum period in the ewe and similarly, Moss et al. (1980) observed no significant changes in hypothalamic GnRH content in ewes on the day after parturition compared with the ewes on day 40 post-partum when ovarian cycles were initiated. Likewise, no change in the concentration of hypothalamic GnRH during the post-partum period were observed in beef cows (Moss et al., 1985; Cermark et al., 1983) or in dairy cows (Carruthers et al., 1980). However, Brever et al. (1962) observed that hypothalamic GnRH content was higher in suckling than in cycling cows, suggesting that GnRH secretion had been inhibited during the post-partum period, resulting in increased stores of GnRH in the hypothalamus. From these observations it is possible to conclude that during the post-partum period in ruminants the hypothalamus contains enough GnRH to stimulate the anterior pituitary gland, but that GnRH secretion may be inhibited. Smith (1978) reported that in rats, GnRH concentrations in the hypothalamus are lower in females nursing eight pups than in those nursing two pups.

In the sow, Cox and Britt (1982a) observed that the hypothalamic stores of GnRH were lower at weaning that at any time during the weaning to estrus interval. However, no information is available on the changes in GnRH activity during different phases of lactation in the sow. It is therefore still unknown in the sow whether the progressive increase in LH levels in peripheral blood during lactation is due to an increasing release of GnRH and/or an increasing sensitivity of the pituitary gland to GnRH stimulation.

### 1.4 Pituitary GnRH receptors during lactation

In the ewe it has been observed (Moss et al., 1980) that the numbers of receptors for GnRH in the anterior pituitary did not differ with time after parturition. However, these observations were slightly different from those in the study of Crowder et al. (1982), who found that the number of GnRH receptors was higher on days 1 and 11, than on days 22 and 35 after parturition. Similarly in beef cows, Moss et al. (1985) found that the numbers of GnRH receptors were reduced at 20 and 30 days after parturition when compared to days 5 and 10. An interesting observation is that in ovariectomized ewes treated with estradiol benzoate, either alone or with progesterone, pituitary GnRH receptors are increased (Moss et al., 1981). In the hamster (Adams and Spies, 1981) there is an increase in the number of GnRH receptors in the pituitary during proestrus, a time when concentrations of estrogen in plasma are elevated.

In conclusion, these data suggest that the elevated levels of estradiol during pregnancy induce a high number of GnRH receptors, while the number of GnRH receptors are diminished during lactation due to the presence of low plasma estradiol-17beta concentrations.

### 1.5 Effect of exogenous GnRH treatment on gonadotropin secretion during lactation

The administration of small doses of GnRH at one or two hourly intervals, to stimulate the pattern of LH secretion which occurs during the follicular phase of the estrous cycle, has induced reproductive activity during post-partum anestrus in domestic animals (Riley et al., 1981; Rojanashien et al., 1987; Bevers et al., 1981; Britt et al., 1985; De Rensis et al., 1991). The release of LH in response to GnRH is diminished during the early post-partum period and does not return to the level seen during pregnancy until 16-25 days post-partum in women (Miyake et al., 1978, Keye and Jaffe, 1976), 7-10 days post-partum (Fernandes et al., 1978; Kesler et al., 1977) or 20 days post-partum (Webb et al., 1977) in the cow, or 3-5 weeks post-partum in the ewe (Jenkin and Heap, 1974; Jenkin et al., 1977).

Although stimulation with GnRH will release sufficient LH to induce ovulation in the dairy cow before day 14 post-partum (Britt et al., 1974; Carter et al., 1980), the pituitary content of LH prior to day 30 in the post-partum beef cow may be insufficient to allow normal ovulation and to maintain luteal function for the duration of a normal estrous cycle, resulting in silent estrus and a short cycle (Lisham et al., 1979). Moreover Evans (1980) reported that the mean level of LH released in post-partum ewes after the injection of GnRH between day 4 and 17 of lactation was only half that observed in treated cyclic ewes. Jagger et al., (1987) treated suckled beef cows between 9 and 26 days of lactation with continuous or repeated administration of GnRH. They noted that although both treatments induced preovulatory-type gonadotropin surges in half the animals, neither treatment regimen overcame anovulation in all cows.

In the lactating sow the injection of GnRH results in LH release (Van de Wiel et al., 1978; Stevenson et al., 1981b). In the pig (Bevers et al., 1981), as in sheep (Kann et al., 1978; Jenkin et al., 1977) and cattle (Schallenberger et al., 1982,b), the LH response to GnRH treatment is reported to be lower immediately after parturition than in late lactation. However, Rojanasthien et al. (1987) measured the LH response to different doses of GnRH during early (days 7-15) and late lactation (days 20-28) in primiparous sows and observed that although the total LH response increased with increasing doses of GnRH during early as well as late lactation, there was no apparent change in the responsiveness with time post-partum. These results are in agreement with those of Stevenson et al. (1981) who did not find any difference in the LH response to GnRH between days 10 and 20 of lactation.

Cox and Britt (1982,b) were the first to demonstrate that fertile estrus could be induced in multiparous lactating sows by pulsatile administration of GnRH on day 25 post-partum. Also Ramirez et al. (1985b), giving hourly pulses of GnRH to 10 primiparous sows beginning on day 24 of lactation, were able to induce the onset of estrus five days after the initiation of GnRH pulses in all the animals treated. More recently, De Rensis et al. (1991) demonstrated effective release of LH episodes in response to pulsatile GnRH administration, associated with development of estrogenic follicles.

In conclusion therefore, it seems that it should be possible to induce estrus during lactation through the administration of GnRH and that better results are obtained when pulsatile administration of GnRH is used and animals are treated in late lactation. However, there considerable variability in the response to treatment. This may be due to pituitary disfunction, seen as a lack of responsiveness to the GnRH stimulation in some animals, even when treated with a pulsatile administration of GnRH in late lactation. This situation probably does not result from a lack of GnRH receptors on the gonadotropes, but may be associated with a low releasable pool of LH in the pituitary during the post-partum period. In turn, this could be a consequence of the block of GnRH release during pregnancy due to high levels of estradiol and progesterone and therefore a lack of LH synthesis. The "escape" of the hypothalamic-pituitary axis from the block due to the suckling stimulus, and the consequent replenishment of pituitary LH content that occurs in late lactation, allows a better response by the hypophysial-ovarian axis to treatment with GnRH. However, in the sow, there are still insufficient data describing the synthesis and release of GnRH, changes in pituitary receptors for GnRH and the response of the pituitary to treatment with GnRH during early lactation. It will be interesting to further study the pulsatile injection of GnRH in the sow because this seems the most effective way to induce follicular development and ovulation during lactation. Alternatively, as discussed later, a means of blocking the inhibitory effects of suckling on the GnRH pulse generator should be effective in stimulating ovarian activity during lactation.

## 2. MECHANISMS INVOLVED IN CENTRAL REGULATION OF GONADOTROPIN SECRETION

The hypothalamus has extensive efferent and afferent connections with various brain structures and many (principally in rodent) experiments have shown that these have both inhibitory and facilitatory effects on gonadotropin secretion. There are many multisynaptic stimulatory and inhibitory pathways between extra-hyphothalamic areas and the hypothalamus and the different neurotransmitters involved include adrenaline, noradrenaline, dopamine, acetylcholine, histamine, serotonin, endogenous opioid peptides (EOPs), N-methyl D-aspartate (NMDA), gamma-amminobutyric acid (GABA) and neuropeptide Y (Krulich, 1979; Elde and Hokfelt, 1979; Bicknell, 1985). Those systems seldom work in isolation but very often operate in concert to determine peripheral LH concentrations. In the following chapter a simplified review of the major neurotransmitters involved in gonadotropin release will be presented.

### 2.1 Adrenaline and noradrenaline

Monoamine regulation of gonadotropin secretion is complex and extensive studies have identified stimulatory or inhibitory effects of adrenaline and noradrenaline on GnRH and therefore on LH release. In the rat the anatomical distribution of noradrenergic neurones has been demonstrated to be in close proximity to GnRH neurones (Alder and Crowley, 1984; Kalra and Kalra, 1984).

In the rat a stimulatory effect of monoamines on GnRH secretion has been reported at least during the LH surge (Ramirez et al., 1984). Moreover, it has been also observed that the blockade of adrenergic receptors is able to suppress GnRH pulsatile release (Drova and Gallo, 1977). Finally, the administration of alpha-adrenergic blockers and inhibition of noradrenaline synthesis can prevent the rise in plasma LH in response to naloxone injection (Karla et al., 1981).

In the pig, Parvizi et al. (1976) observed a decline in LH secretion in boars receiving intrahypothalamic injections of noradrenaline. The administration of exogenous noradrenaline has also been reported to inhibit LH secretion in ovariectomized ewes (Deaver and Dailey, 1982) but to enhance secretion in anestrous ewes (Przekop et al., 1975). Furthermore Meyer and Goodman (1985), using an intravenous infusion of an adrenergic blocker, Phenoxybenzamine in seasonally anestrous ewes, observed an inhibitory effect of noradrenaline on LH secretion. From those data therefore it appears that noradrenaline has an excitatory effect on LH secretion in presence of estrogen, while in absence of estrogen the effect is inhibitory.

Using combined treatment with noradrenaline and opioid agonists and antagonists (Diez-Guerra et al., 1986; Kalra et al., 1981; Dyer et al., 1988) it has been demonstrated that many of the effects of the noradrenergic systems on the GnRH secretion are associated with the opioidergic system and that the nature of this interaction is dependent on the presence (stimulatory) or absence (inhibitory) of estrogen. It is interesting to note that α-adrenergic blockers and noradrenaline synthesis inhibitors will prevent the rise in plasma LH in response to naloxone injection, suggesting that endogenous opioids act to suppress release of noradrenaline (Kalra and Simpkins, 1981). There are reports supporting this suggestion. It has been observed that the endogenous opioids decrease noradrenaline turnover (Alder and Crowley, 1984) and that activation of the adrenergic innervation to the hypothalamus by electrical stimulation of the ventral noradrenergic tract increases plasma LH levels, an effect that can be potentiated by naloxone (Dyer et al., 1985). Furthermore, opiate receptors have been located in those hypothalamic areas in which GnRH cell bodies are found (Hammer, 1985; Lehman et al., 1986). An influence of EOP on the feed-back effect of estradiol 17-beta on GnRH release has also been suggested. In fact Morrel et al. (1985) found that in the rat hypothalamus some endorphin and dynorphin-containing neurones may concentrate estrogen.

Thus an opioidergic influence on GnRH neurones may be regulated by gonadal steroids acting on opioidergic neurones. In support of this, it also been reported in the human gonadal steroids have also been reported to alter the hypothalamic content of β-endorphin (Wardlaw et al., 1982). Thus, alteration of the opioid-adrenergic input to GnRH neurones may be part of the mechanism by which gonadal steroids exert their central feed-back effects on gonadotropin secretion. Opioidergic regulation of GnRH neurones therefore appears to form part of the physiological regulatory mechanism governing gonadotrophin secretion, during both pulsatile GnRH release responsible for maintaining "basal" plasma LH levels in both male and female rats, and the preovulatory gonadotropin surge in females (Kalra and Kalra, 1984; Dyer, 1988).

In conclusion, from the data reported above it seems possible to suggest that the stimulatory or inhibitory effects of adrenaline and noradrenaline depend on the reproductive stage considered and are also considerably influenced by the opioidergic systems.

## 2.2 Dopamine

Regarding the relationship between dopamine and GnRH, some in vitro studies indicate that dopamine can stimulate the release of GnRH and thus LH (Rasmussen et al., (1986). Also in vivo it has been demonstrated that a dopaminergic antagonist (pimozide) consistently blocked the estradiol-induced LH surge and suppressed LH secretion in ovariectomized ewes (Jackson et al., 1977). Sneider and McCann (1970) report that dopamine is only effective in enhancing LH in steroid treated ovariectomized females. Similarly, it was demonstrated that in vitro release of LHRH from incubations of the mediobasal hypothalamus was only enhanced by dopamine when the tissue was taken from intact male or ovariectomized, estrogen-treated female rats (Rotsztejn et al., 1976). Collectively, these data suggest that the dopaminergic stimulation of LHRH is apparent only in intact or steroid treated castrate animals. Furthermore, it has been shown that the catecholamine content of tuberoinfundibular cell bodies exhibit a cyclical variation throughout the estrous cycle of the rat, confirming that the ovarian steroids are important in determining the influence of the dopaminergic system on LH secretion (Ahern et al., 1978; Barraclough et al., 1982). Consistent with the evidence for a stimulatory effect of dopamine on the central control of GnRH and LH secretion, treatment with the dopamine agonist bromocriptine restored ovulatory function in women with hyperprolactinaemia (Besser et al., 1972; Thorner et al., 1975; Del Pozo et al., 1974) and increased mean LH concentrations in lactating sows (Bevers et al., 1981). There are, however, many experiments that suggest an inhibitory, rather than a facilitatory role, for dopamine on LH release (Miyachi et al., 1973; Uemura and Kobayaskhi, 1971; Drouva and Gallo, 1976; Beck and Wuttke, 1977; Ramirez et al., 1984; Leebaw et al., 1978). In ewes dopamine inhibited release of GnRH from the median eminence (Kuljis et al., 1989) and a fall in plasma LH concentration after bromocriptine treatment has been described in the sows (Kraeling et al., 1982). Furthermore, results based on the treatment of luteal phase ewes with dopamine or a dopamine receptor antagonist, suggest that progesterone might exert an inhibitory effect on LH secretion through a dopaminergic mechanism (Deaver and Dailey, 1983; Deaver et al., 1987, Dailey et al., 1987). Also in intact anestrous sheep, dopaminergic antagonists (phenoxybenzamine and pimozide) increased pulsatile secretion of LH (Meyer and Goodman, 1986) and it appears that a dopaminergic inhibitory effect on LH secretion predominates in anestrous ewes. Exquisite sensitivity to ovarian hormone feed-back, probably estradiol (Goodman et al., 1980, 1982), may regulate the activity of this inhibitory neural system and thereby suppress LH pulse frequency. Finally, acute administration of bromocriptine in normal human subjects has been reported to have no effect on LH secretion (Tolis et al., 1975, Evans et al., 1980; Mattioli et al., 1986).

The overall conclusion from these apparently contradictory data is that some effects of dopamine on GnRH are mediated by the presence of ovarian steroids; thus, as Dailey et al. (1987) suggest, the different actions of catecholamine antagonists in the different species, with and without ovaries, could be due to the relative activity of two different catecholaminergic systems (dopaminergic and noradrenergic) involved in regulating episodic secretion of LH.

The specific effects of dopamine on gonadotropin secretion depends on the type of dopamine receptors present at each level of the brain-hypothalamo-hypophysial axis. As many as five different dopamine receptors have been identified (Sokoloff et al., 1990; Dearry et al.,1990; Sunahara et al.,1991) and Kebabian and Calne (1979) first proposed that dopamine receptors in the brain are divided into two separate subtypes designated D1 and D2. They differ in a variety of aspects, including molecular weight, coupling to adenylate cyclase, location and affinity for dopamine agonists and antagonists. The D1 but not the D2 receptors are positively coupled to the adenylate cyclase enzyme (Creese et al.,1983). Furthermore dopamine receptors exist pre- and post-synaptically. Recently Sokoloff et al. (1990) have been able to characterize a receptor subtype D3, which differs in its pharmacology and signalling system from D1 and D2 receptors and, as for D2 receptors, seems to be both an autoreceptor and a postsynaptic receptor. The D3 receptor is localized in the limbic areas of the brain associated with cognitive, emotional and endocrine function. However, it seems the dopaminergic neurones of the tuberoinfundibular system do not posses the 'auto-receptors' characteristic of the other dopaminergic systems (Moore and Denarest, 1982). Finally, James et al. (1987) observed in the rat that the selective D2 agonist LY171555 and D2 antagonists, sulpiride and domperidone, had no effect on plasma LH or ovulation, but the D1 agonist SKF 38393 stimulated LH release, indicating that the stimulatory effect of dopamine on LH release is centrally mediated via D1 receptors.

There are some differences between studies in the apparent site of dopaminergic control of gonadotropin secretion. However, overall, the main site of action of dopamine on gonadotropin secretion is generally believed to be at hypothalamic level on GnRH neurons. These conclusions have been also drawn from observations that incubation of pituitary cells with dopamine in vitro failed to affect LH secretion (McCann, 1983). Other studies suggest a pituitary site of action for dopamine modulation of LH release and this is supported by the identification of pituitary dopamine receptors in various species (Nunez et al., 1981; Johns et al., 1982). In teleost fish, dopamine acts directly on the gonadotropes to decrease the binding of GnRH (DeLeew et al., 1988) and dopamine receptors have been identified on a subpopulation of gonadotropes (Goldsmith et al., 1979). Other studies of dopamine receptor distributions in mammalian species indicate that only D2 receptors are found in the pituitary (Thomas et al., 1989), whereas in the median eminence receptors are primarily of the D1 type (Leibowitz et al., 1982). Others have reported that there are also D1 receptors in pituitary cells and administration of Fenodolpan (a specific dopamine D1 agonist) resulted in a dose-dependent decrease in PRL secretion in vivo and in vitro in the rat (Schoors et al., 1991). In a recent study Martinez-de-la-Escalera et al. (1992) and Findell et al. (1993) report that there is a direct stimulatory effect of DA on GnRH release via dopamine D1-receptors but not dopamine

D2-receptors.

By infusing Fenodolpan during the follicular phase in woman, Boesgaard et al. (1991) induced an increase in the LH and FSH responses to GnRH administration, and also suggested that this dopamine agonist was acting at the hypophysial level. The release of LH in response to GnRH is decreased in ovariectomized, pituitary stalk-transected ewes (Donnelly and Dailey 1991), in stalk sectioned rabbits (Dailey et al., 1978), ovariectomized ewes (Deaver et al., 1982) and humans (Leebaw et al., 1978) treated with dopamine infusion.

In conclusion, although there are conflicting data in vivo and in vitro on dopamine actions on LH release, it seems possible to postulate that dopamine modulates gonadotropin secretion by regulating GnRH secretion at the hypothalamic level. However, a pituitary effect of dopamine cannot be excluded. The presence of either inhibitory or stimulatory effects of dopamine agonists and antagonists suggests that other regulatory systems are involved in modulating the effects of dopamine on GnRH and LH secretion during different reproductive periods.

### 2.3 Serotonin

The precise role of serotonin on LH secretion is not well defined. An inhibitory effect of serotonin on basal LH secretion (Kordon, 1968) and a block of the preovulatory surge of LH in cyclic ewes (Domanski et al., 1975) has been reported. In contrast to evidence for an inhibitory role for serotonin, Deaver and Dalley (1982) and Donnelly et al. (1992) reported that the administration of exogenous serotonin greatly increased LH secretion in ovariectomized ewes. However, both the stimulatory and inhibitory role of this neurotransmitter appeared in some doubt, as cyproheptadine, a serotonergic antagonist, was demonstrated to have no effect on LH secretion in anestrous and cycling ewes (Meyer and Goodman, 1986).

### 2.4 Endogenous opioid peptides

In recent years several comprehensive reviews on the opioids have been published (Bicknell, 1985; Brooks et al., 1986; Malven, 1986; Haynes et al., 1989; Barb et al., 1991; Britt et al., 1993; Schillo, 1993; Rawlings et al., 1993; De Rensis et al., 1993a) and Table 1 summarizes research conducted in domestic species using the EOP antagonist naloxone. The family of peptides comprising the endogenous opioids is divided into three sub-groups by virtue of structure and precursor molecule. The enkephalins, beta-endorphins and dynorphins exhibit varying affinity for at least five classes of opiate receptors, including mu, kappa, delta and sigma. Heterogeneity in receptor binding remains a principal obstacle in assigning specific responses to particular opioids (Crowley, 1986).

There are two levels at which opioids may modulate gonadotropin secretion. Firstly they may interfere with GnRH action at the anterior pituitary gland; secondly they may influence GnRH secretion within the hypothalamus. Several lines of evidence argue against the hypothesis that opioids inhibit LH secretion via direct actions upon the anterior pituitary. Early in vivo and in vitro studies in a variety of species showed that neither naloxone nor morphine altered the pituitary LH responses to pulses of GnRH (Cicero et al., 1977; Grossman et al., 1989; Ferin et al., 1984; Ebling et al., 1987) and naloxone did not disrupt the secretory response of the pituitary to exogenous GnRH given in vivo (Cicero et al 1977; Grossman et al., 1984, Ferin et al., 1984). Furthermore in the rat, GnRH antagonist treatment decreased naloxone-induced elevations in plasma LH (Blank et al., 1982) and morphine reduced secretion of GnRH into pituitary portal plasma (Ching, 1983). Finally, in the sheep there is no direct pituitary action of morphine on LH secretion in vivo and no specific binding in the anterior pituitary gland (Horton et al., 1990). However, other authors have reported effects of opiates at the pituitary level (Chao et al., 1986; Blank et al. 1986; Mattery and Moberg, 1985; Chao et al., 1986, Barb et al., 1990). Therefore it seems that opioids may have a more important role in the control of LH secretion at the pituitary level than was suggested from earlier studies.

The precise site in the central nervous system at which opioids might affect GnRH secretion remains largely speculative. On the basis of several observations it seems that opioids may act in the medial preoptic area, the median eminence and the arcuate nucleus (King et al., 1982; Witkin et al., 1982; Kalra, 1981, 1983). The highest concentrations of beta-endorphin in the rat were reported to be in the medial hypothalamus, with lower concentrations in the thalamus, zona compacta of the substantia nigra, medial amygdaloid nucleus, periacqueductal grey area and the locus coeruleus (for review see Morley, 1981; Grossmann et al.,1989). Hypothalamic beta-endophinergic neurones originate largely in the arcuate nucleus from where they project to the median eminence as well to other parts of the hypothalamus and brain (Howlett et al 1987).

Although there is no available evidence on whether LHRH neurons are innervated directly by any of the opioidergic systems (for review see Hoffman et al., 1989), the above data and the stimulatory effect of naloxone on LHRH release under in vitro conditions (Blank et al., 1982; Leadem et al., 1985) demonstrate that opioids affect the release of LHRH from the hypothalamus. Furthermore, both the corticotropes in the anterior lobe and the melanotropes in the intermediate lobe of the pituitary gland contribute to the total secretion of beta-endorphin into the peripheral circulation (Smith and Funder, 1988).

Although different classes of opioid receptors have been described (Paterson et al., 1983; Herz et al.,1989), the mu receptors appear to be particularly involved in the control of gonadotropin secretion (Cicero et al., 1983; Pfeiffer et al., 1983; Panerai et al., 1985; Laedem et al., 1985;). However, there are suggestion also for action via kappa receptors (Goodman et al., 1980; Pfeiffer et al., 1983) and the studies of Wiesner et al., (1985) indicate that delta but not mu receptors are implicated in opioid inhibition of LH. Finally Hoffman et al. (1989) reported that only a fraction of GnRH cells are contacted directly by opioidergic neurones.

### 2.4.1 The opioids and post-partum anestrus in domestic animals.

#### Cow

In cattle, LH pulse frequency and therefore GnRH secretion is depressed during the post-partum period and this suppression exists for a greater length of time in suckled compared to milked cows, implying that the suckling stimulus rather than lactation itself was responsible for LH suppression (Peters et al., 1981).

One of the mechanisms by which suckling inhibits GnRH secretion in the cow involves the opioids. Naloxone infusion into suckled beef cows around day 40 post-partum resulted in a significant increase in LH pulse frequency and amplitude, while naloxone was ineffective in raising LH in non-suckled post-partum cows (Whisnant et al., 1986a,b). Calf removal usually abolished the response to naloxone. It has also been shown that a larger dose of naloxone is required to elicit an LH response on day 14 post-partum compared to days 28 and 42, leading to the proposal that opioidergic inhibition of LH in the post-partum cow appears to be more potent in the early post-partum period (Whisnant et al 1986b). It would therefore appear that the removal of the suckling stimulus disinhibits the effect of opioids on LH secretion in the cow. Cross et al. (1987) observed that naloxone increased mean LH concentration, and episodic LH pulse frequency and amplitude in suckled beef cows at day 18-19 post-partum but a response was also evident three days after weaning at day 24. From the above data and the observations of a latent effect on the pituitary of the high steroid levels during gestation (see Nett, 1987), it seems possible to conclude 1) that in cows, suckling influences LH secretion at the later but not during the immediate post-partum period and 2) that the opioids in cows are effective in inhibiting GnRH secretion but this effect gradually diminishes during the post-partum period.

### Sheep

Research in the sheep is difficult to relate to that in other domestic species due to marked reproductive seasonality. However, during the post-partum period in the ewe, naloxone significantly increases LH concentration, with a decrease in the response of LH to naloxone treatment with time from lambing (Gregg et al., 1986).

Elevations in post-partum LH concentrations following EOP antagonism were associated with depressions in PRL secretion in suckled and non-suckled ewes. In another study, involving the alternative approach of direct estimation of hypothalamo-hypophysial beta-endorphin concentrations and receptor populations, there was a suggestion that under the stimulus of suckling, this opioid exhibits an autocrine role within the hypothalamus, inhibiting GnRH release and also the dopaminergic suppression of PRL secretion (Gordon et al., 1987). Hence, plasma PRL concentrations may represent the result of hypothalamic EOP inhibition rather than playing a causal role in GnRH regulation. In the spring lambing ewe, naloxone was administered to suckling ewes and to ewes weaned 20 days post-partum (3 days after lamb removal). EOP antagonism elevated LH secretion in both groups when compared with non-suckled and suckled counterparts without EOP inhibition. These results suggest that although EOP may have reduced gonadotropin secretion in these animals, their effects were not modified by suckling (Newton et al., 1988). It is noteworthy, however, that LH concentrations in the control, weaned group fell following weaning when compared with suckled ewes. The possibility remains that suckling effects were still evident 72 hours post-weaning and the authors argue that, although a comparison between weaned and weaned plus naloxone was unobtainable, suckling was unlikely to have affected responses to the antagonist. On this basis, they suggest that the post-partum lactating ewe differs from species exhibiting a distinct lactational anestrus, e.g. pigs and cattle.

Thus in the ewe the opioidergic suppression of LH did not appear dependent on suckling, since the response to naloxone is still present in ewes from which the lambs are removed at day 10 of lactation and there are no differences in pre-naloxone LH values between suckled and non-suckled ewes. Thus it is not surprising that there are no differences in the time from lambing to the first post-partum estrus between suckled and non suckled ewes (Fletcher et al., 1973; Moss et al., 1980).

In conclusion, it seems that although opioids reduce gonadotropin secretion in the sheep, this effect is independent of the suckling stimulus.

### Pig

Suckling in the sow has been shown to inhibit LH secretion by a suppression of the GnRH pulse generator, as evidenced by a LH response to a single GnRH challenge (Rojanasthien et al., 1987) and to repeated GnRH injections during suckling, (Bevers et al., 1981; Britt et al., 1985; De Rensis et al., 1991). The principal cause of lactational anestrus is the inhibitory effect of suckling on hypothalamic GnRH release and the resulting decline in episodic LH secretion. The opioids have been shown to be prime candidates as mediators of this inhibitory effect of the suckling stimulus on LH secretion. Barb et al. (1986a) and Mattioli et al. (1986) first observed that naloxone injections are able to increase LH plasma during lactation in swine. Armstrong et al. (1988a) also blocked the increase in LH secretion associated with transient weaning and demonstrated a delayed estrus onset in weaned sows following morphine administration. Armstrong et al. (1988b) also report that constant infusion of naloxone in suckled sows, while increasing LH pulse frequency, failed to elevate mean LH levels above those of suckled sows treated with saline, whilst temporary removal of the litter (for 8 hours) caused a significant increase in both LH mean and LH pulse frequency.

In summary, in the pig the opioids appear to play a role in post-partum control of LH secretion and therefore in lactational anestrus, although their role appears to vary with time post-partum.

### 2.4.2 Peripheral endorphin and GnRH secretion

The possibility that peripheral plasma endorphin can influence GnRH\LH secretion has been poorly investigated. In the sheep beta-endorphin in the peripheral circulation is assumed to be secreted primarily from the pituitary gland, since this tissue contains large amounts of beta-endorphin immunoreactivity compared with the hypothalamus (Ebling et al., 1987).

In lactation it has been established that suckling induces an increase in peripheral concentrations of beta-endorphin in women (Franceschini et al., 1989), rats (Riskind et al., 1984) and sheep (Gordon et al., 1987). Furthermore, Franceschini et al. (1989) observed that in lactating women the increase in plasma beta-endorphin occurred independently of plasma cortisol variations and they suggested that beta-endorphin secreted during suckling is derived from an extrahypothalamic site, i.e. the gut. Plasma endorphin can also have an effect at the hypothalamic level because in the human endorphins can cross the blood brain barrier (Gener et al., 1982). The content of beta-endorphin in the hypothalamus 12-18h post-partum is elevated compared to 6 to 8 days after parturition in the rat (Wardlaw et al., 1983). Finally, in photoperiodically sensitive species, beta-endorphin concentrations can change with season. A marked seasonal cycle in the peripheral blood plasma concentrations of beta-endorphin occurs in sheep, with a 10- to 20- fold increase from spring to autumn (Ebling and Lincoln, 1985).

### 2.5 Other neurotransmitters

Gamma-amino butyric acid (GABA) secretory neurones have been described to be in contact with GnRH neurones (Leranth et al., 1986). The administration of GABA in rats induces GnRH inhibition (Masotto et al., 1987) and this effect is mediated by the noradrenergic system (Masotto et al., 1989). In addition to its central neuroendocrine effects it has been observed that GABA can inhibit LH secretion at the pituitary level (Lux-Lantos et al., 1992). A second excitatory amino acid that has a stimulatory effect on GnRH secretion is N-methyl D-aspartate (NMDA)(Gay and Plant,1987) and an interaction with the opioidergic system has been also suggested for this neurotransmitter (I'Anson et al., 1989). Finally neuropeptide Y is implicated in mediating the feed-back effect of gonadal steroids on LHRH secretion (Weiner et al., 1988), with an inhibitory effect in castrate rats (Karla and Crowley, 1984) and rabbits (Khorram et al., 1987) and a stimulatory effect in intact or steroid treated rats (Kalra and Crowley, 1987).

### 2.6 Interactions between opioids, dopamine and gonadotropin secretion

In laboratory animals the opioids have been shown to inhibit (Pang et al., 1977; Bhanot and Wilkinson, 1984), stimulate (Bhanot and Wilkinson, 1984) or have no effect (Ieiri et al., 1980) on FSH secretion. In adult ewes, naloxone and morphine did not influence FSH secretion (Currie and Rawlings, 1987, 1989) while an effect of naloxone on FSH secretion was evident in 10 week old lambs (Rawlings et al., 1991). Reports on an endogenous opioid involvement in the control of FSH secretion in the pig are limited. Trudeau et al., (1988, 1989) reported that in young male pigs morphine suppressed, whereas naloxone increased, FSH levels and these effects were dose dependent. In contrast, Prunier et al. (1990) and Cosgrove et al. (1991) have observed a suppression of LH secretion but no effect on FSH secretion following naloxone administration to 10-week old males or pre-pubertal gilts.

Available data suggest that the mechanisms by which opioids suppress LH secretion may not involve the dopaminergic system. In fact the combined treatment of post-partum ewes with metoclopramide (a dopamine antagonist) in addition to naloxone did not modify the response of LH (Knight et al., 1986), leading to the conclusion that endogenous opioids suppressed LH secretion in the suckling ewe by a mechanism insensitive to dopaminergic modulation, as previously proposed by Kann et al. (1979). However the release of GnRH from medio-basal hypothalamic fragments, induced by KCl depolarization or DA, can be inhibited by submicromolar concentrations of met-enkephalin, leu-enkephalin or beta-endorphin (Drouva et al., 1980; Rotsztejn et al., 1978a; Rotsztejn et al., 1978b; Drouva et al., 1981).

In the post-partum sheep elevations in LH concentrations following opioid antagonism on days 10 and 26 post-partum, (Gregg et al., 1986) are associated with depressions in PRL secretion in suckled and non-suckled ewes. In another study involving the alternative approach of direct estimation of hypothalamo-hypophysial beta-endorphin concentrations and receptor populations, there was a suggestion that under the stimulus of suckling, the opioids exhibit an autocrine role within the hypothalamus, inhibiting GnRH release and the dopaminergic suppression of PRL secretion (Gordon et al., 1987). However, there are studies indicating that dopamine can influence opioid secretion and might therefore exert indirect effects of GnRH and LH secretion. In humans, a tonic dopamine inhibition of beta-endorphin was reported (Genazzani et al., 1984) and stimulatory effects of DA antagonists on beta-endorphin secretion have been observed in rats (Farah et al., 1983; Giraud et al., 1980)and in dogs (Sharp et al., 1982). Furthermore Vermes et al. (1984) demonstrated that the release of beta-endorphin from the mediobasal hypothalamus in vitro is under the inhibitory control of dopaminergic neurons. In the ram dopamine inhibits beta-endorphin and PRL secretion under either long or short days and Ssewannyyana et al. (1990) reported that circulating concentrations of beta-endorphin and PRL decrease following treatment with bromocriptine during different stages of the photoperiod-induced cycle.

Recently it has been reported that mu receptors undergo upregulation in response to chronic cocaine exposure, suggesting that dopamine activity can regulate the expression of mu opioid receptors (Unterwald et al., 1992). Dopamine receptor agonists (bromocriptine, lysuride) do not change plasma beta-endorphin when acutely administered but induce responses during chronic treatment (Autelitano et al., 1987). These results further confirm that there is a chronic and not an acute effect of dopamine on GnRH\LH secretion.

## 3. THE GONADOTROPINS AND MECHANISMS MODULATING THEIR SECRETION

### 3.1 Hypothalamic control

LH and FSH are glycoprotein molecules with a molecular weight of about 30,000 and consist of $\alpha$ and $\beta$ subunits. The $\alpha$ subunit is common to all glycoprotein hormones of a given species, whereas the $\beta$ unit confers specific biological properties to the hormone (Vaitukatis et al., 1976). The gonadotropic hormones are produced within the basophilic cells of the anterior pituitary. Following their synthesis they are stored within secretory granules and are later released from the gonadotrope cells by exocytosis (Fawcett et al., 1978). Both gonadotropins are probably metabolized by the liver and kidneys (Hutchinson and Sharp, 1978). Studies involving the injection of labelled hormone in a number of species have estimated the half life of LH at about 28-35 minutes (Geshwind and Dewey, 1968) and the half life of FSH at about 130-160 minutes (McNeilly, 1988).

The pulsatile nature of LH release was initially described in the ovariectomized rhesus monkey (Dierschke et al., 1970) and subsequently in sheep (Foster et al., 1975) and pigs (Foxcroft et al., 1975). It is now widely accepted that during the estrous cycle in all female mammals that have been studied, two predominant modes of LH secretion have been observed: the tonic, episodic pattern and the surge or cyclic pattern. The first is epitomized as a variable pattern of episodic LH release above basal levels; the second, in contrast, operates rather acutely and is responsible for the massive sustained increase in circulating gonadotropin concentrations that causes ovulation (Jackson et al., 1977; Hafez, 1984).

There are situations in which FSH and LH are differentially secreted. For example a peak of FSH secretion is coincident with the preovulatory surge of LH, both of which are induced by estradiol. However, a second major increase in FSH is observed in most species commencing 18-24h after onset of the preovulatory release of both gonadotropins. This increase reaches peak concentrations just after ovulation when LH and estradiol concentrations are both low (Baird and McNeilly, 1981). In the rat, the peak of the spontaneous FSH surge occurs about 11h after the spontaneous proestrous LH surge (Aiyer et al., 1974a) and dissociation between LH and FSH secretion also occurs during sexual development (Chiappa et al., 1977) and in response to experimental manipulation of sex steroids (Ayer et al., 1974b). Clear-cut dissociation between LH and FSH secretion in women also occurs during the follicular phase of the menstrual cycle and during pregnancy (Parlow et al., 1970), as well as after the administration of estradiol (Nillius et al., 1971). In the sow, circulating concentrations of LH are low during the early post-partum period and remain generally suppressed during the first three weeks of lactation, while the concentrations of FSH are not suppressed and shown only little change (Walters et al., 1982; Moss et al., 1985; Lauderdale et al., 1965; Stevenson et al., 1981b). At weaning LH, but not necessarily FSH, concentrations increase significantly (Shaw and Foxcroft 1985). Secretion of FSH and LH are both abolished after administration of a centrally active compound, methallibure (synonymous with AIMAX or ICI 22828) (Kesner et al., 1987) and after passive immunization against GnRH (Esbenshade and Britt, 1985), but hypophyseal stalk transection induces a decrease of LH (Carpenter and Anderson, 1985; Kraeling et al., 1986; Kesner et al., 1989) but not FSH (Kraeling et al., 1986).

There are many external factors known to influence FSH and LH secretion. For example in sheep, the relative concentrations of the two gonadotropins have been related to breed, sex, nutrition, season and age. In all these instances, it is apparent that the secretion of FSH can change independently of LH, indicating that the control systems regulating the two gonadotropins are not necessarily the same. Although there have been reports postulating the existence of separate LH and FSH releasing hormones (Bowers et al., 1973; Folkers et al., 1978), the two releasing hormone concept does not appear to be an adequate explanation for differential regulation of LH and FSH secretion. There is instead more evidence for a difference in the secretion of FSH and LH due to the inherent properties of pituitary cells. Removal of hypophysial cells from the influence of the hypothalamus (Clarke et al., 1983) or GnRH in vivo (Clarke et al., 1984) or in vitro (Miller et al., 1977), results in a slower decrease in the secretion rate of FSH compared to LH. The dissociation between LH and FSH secretion may be dependent upon the pattern of exposure of the pituitary gland to GnRH. Clarke et al. (1986) observed that LH but not FSH secretion ceases when constant GnRH or saline infusions are given to ovariectomized and hypothalamic-pituitary disconnected ewes and suggest that FSH synthesis, but not FSH secretion, requires pulsatile GnRH release. Similarly, Brown-Grant and Greig (1975) showed that in the rat the spontaneous LH surge could be blocked immediately and completely by the injection of sodium pentobarbitone at any time during the rising phase of the surge, but, once established, the FSH surge was not interrupted by the injection of sodium pentobarbitone. In the human it also appears that, once established, FSH release continues for much longer than LH release (Yen et al., 1972). These observations suggest that the secretion of LH, but not FSH, is dependent upon the minute-by-minute exposure of the pituitary gland to GnRH. Once FSH secretion is initiated by GnRH, it may continue to be secreted, perhaps autonomously, at least for several hours.

The gonadotropes, as do other anterior pituitary cells, have similar,if not identical, biochemical pathways involved in the regulation of hormone release. These pathways include interactions among phospholipid and arachidonic acid metabolites, calcium mobilization, cyclic nucleotides and undoubtedly other as yet unidentified systems. Although the mechanisms of secretagogue-induced hormone release are complex, it appears that two general mechanisms can be identified that are responsible for mediating immediate post-receptor events. These include the systems surrounding the hydrolysis of phosphatidilinositol-4,5-biphosphate to inositol triphosphate and diacylglycerol (DAG), or cyclic adenyl nucleotide (cAMP) metabolism (see Fink., 1988b). Therefore, in response to the activation of a pituitary receptor by a GnRH, the secretion of one or other gonadotropin could be dependent on activating different intracellular biochemical pathways. Also steroid feed-back can be involved in the mechanisms regulating differential gonadotropin secretion. In ovariectomized (ovx), estradiol benzoate treated ewes, LH concentrations are below those of untreated ovx ewes (Diekman and Malven, 1973). Estradiol, in combination with progesterone in the correct sequence and doses, can result in changes in LH concentrations in ovariectomized ewes which mimic those of intact, cyclic animals (Goodman et al., 1980a). The situation with FSH is different. Although estradiol has a long-term negative influence on FSH secretion, none of the steroid replacements maintained FSH concentrations in the physiological range (Goodman et al., 198a).

In vitro and in vivo estradiol exerts a biphasic action on LH secretion, initially inhibiting, then augmenting LH responses to GnRH. In contrast, estradiol inhibits FSH release both in vivo and in vitro. (see Clark, 1988). Yen et al. (1971) suggested that estradiol may preferentially promote the release of LH rather than FSH and Miller and Wu (1981) observed that in sheep pituitary cell cultures, estradiol is a highly potent inhibitor of FSH release.

Another mechanism involved in the differential regulation of gonadotropin secretion is the feed-back regulation by inhibin and other non-steroidal factors of FSH secretion (for review see Ackland et al., 1992).

In conclusion, although all the mechanisms involved in the control of LH and FSH are not well understood, it is clear that there are physiologically important differences in the secretion of the two hormones. The differential regulation of LH and FSH can occur at two levels, the hypothalamus and higher brain centres or the pituitary. At the hypothalamic level a specific FSH releasing factor has not been yet identified, although the existence of factors secreted in the brain that can selectively stimulate FSH secretion cannot be excluded. The pattern of GnRH secretion may differently influence the synthesis and secretion of the gonadotropins by differentially regulating gonadotropin subunit mRNA expression. At the pituitary level, there is evidence that the gonadotropes which synthesize and secrete FSH have a much greater capacity to continue this function than those which produce LH. Different intracellular biochemical pathways could also be involved in differential LH and FSH secretion. Finally, ovarian factors are clearly involved in the regulatory mechanisms operating at the pituitary level and inhibin, with a specific inhibitory effect on FSH, is of particular importance.

### 3.2 Ovarian steroid feed-back

It is well recognized that two of the main factors controlling gonadotropin secretion are progesterone and estradiol-17beta secreted by the ovary. Generally, ovariectomy is followed by an increase in plasma gonadotropin concentrations (Martin et al., 1984; Montgomery et al., 1985; Webb et al., 1985). This is a direct effect of removing ovarian hormones as confirmed by the administration of exogenous estradiol in ovariectomized animals (Yamaji et al., 1972; Sarkar and Fink, 1980; Karsch et al., 1980; Goodman et al., 1982; Rawlings et al.,1984). Moreover, Debeljuk and colleagues (1972) reported that estradiol alone or in combination with progesterone produced a suppression of pituitary responsiveness to GnRH in intact diestrous rats.

With regard to farm animals, it has been observed that both estradiol-17beta and progesterone are able to suppress the post-ovariectomy rise in plasma LH concentration in the cow (Beck et al., 1976), ewe (Diekman and Malven, 1973; Baird et al., 1976; Karsh et al., 1977; Tamanini et al., 1986; Martin et al 1984; Webb et al., 1985; Joseph et al.,1992; Currie et al., 1992) and sow (Rayford et al., 1974; Parvizi et al., 1976). Crowder and colleagues (1982) observed that treatment of ovariectomized ewes with a silastic implant containing estradiol-17beta and progesterone caused a reduced response in pituitary LH release after stimulation with GnRH. This was associated with a parallel decrease in the pituitary concentration of LH, while the hypothalamic content of GnRH did not change throughout the treatment period. When the steroid implants were removed, the responsiveness of the pituitary to GnRH increased. Therefore it seems possible to suggest that the feed-back of ovarian steroid hormones has an important influence on pituitary responsiveness to GnRH. However, in the ovariectomized ewe, Goodman and Karsh (1980a) reported that progesterone reduced the frequency of pulsatile LH release, an effect that would be mediated at the hypothalamic level. Additional evidence to demonstrate negative feed-back of estrogen in the pig is provided by the data of Foxcroft and colleagues (1975), who showed that estrogen treatment will inhibit both GnRH induced LH activity and endogenous episodic LH release in prepubertal gilts. However the presence of a positive feed-back effect of the estradiol-17beta on LH secretion has been observed during the estrous cycle. In fact high estradiol-17beta concentrations are the trigger for the preovulatory LH surge in the cow (Short et al., 1979), sheep (Karsh and Foster, 1975) and pig (Lantz and Zimmerman,1972). In various experiments exogenous estradiol injection has been shown to elicit a characteristic release of gonadotropins in the pig (Foxcroft and Elsaesser, 1977; 1978; Edwards and Foxcroft, 1983b), in cattle (Beck and Convey, 1974), in sheep (Goding et al., 1969) and in rats (Ramirez and Sawyer, 1965). In the studies on the effects of estradiol on FSH secretion, depression was suggested (Rawlings et al., 1984).

With regard to plasma concentrations of estradiol-17beta and progesterone during the post-partum period in the sow, plasma progesterone decreases sharply around the time of parturition and remains low throughout lactation (Ash and Heap, 1975; Baldwin and Stabenfeldt, 1975; Parvizi et al., 1976). There is an increase in plasma estradiol (Robertson and King, 1974; Ash and Heap, 1975) concentrations during farrowing and then plasma concentrations fall rapidly after parturition and remain low (Ash et al.,1973; Edquist et al., 1974; Ash and Heap, 1975; Rojanasthien et al., 1987) or fluctuate (Stevenson et al., 1981b; Pope, 1982; Watson et al., 1987; Varley and Foxcroft, 1990) during lactation.

It has been reported that suckling changes the sensitivity of the hypothalamic-pituitary axis to the estradiol feed-back and both an inhibitory and a stimulatory effect on LH release has been described in various species (Saesser et al., 1973; Foster, 1979; Elsaesser and Parvizi, 1980; Wright et al., 1981; Peters, 1984; Garcia-Winder et al., 1986; Watson, 1987, Clarke, 1987; Wilson et al., 1993) and in vitro (Tang and Spies ,1975; Drouin et al., 1976; Labrie et al., 1978; Padmanabhan et al., 1978).

In the dairy cow the positive feed-back of estradiol-17beta on LH is absent shortly after parturition but is restored by day 15 (Schallenberger et al., 1982,a). Similarly Peters (1984) found a progressive recovery of the positive feed-back mechanism between days 10 and 17 of lactation in beef cows implanted with estradiol benzoate. In cows Watson (1987) measured the concentration of estradiol-17beta between day 21 and 27 post-partum and found that the mean amplitude of any fluctuations in estradiol-17beta was significantly greater in the cows which ovulated earlier. They concluded that the amplitude of the fluctuation in estrogen concentration appeared to be more important than the mean concentration itself in determining whether ovulation was initiated. Finally, in the ovariectomized lactating cow, chronic administration of exogenous estradiol in low concentrations has a positive feed-back effect on the secretion of LH (Garcia-Winder et al.,1986).

In the ewe, there is conflicting data concerning the feed-back effect of estradiol on the hypothalamic-hypophysial axis during lactational anestrus. In fact, while Foster (1979) reported that the post-partum anestrus in the ewe is not mediated by hyper responsiveness to estradiol negative feed-back, Wright and colleagues (1981,b) reported that in post-partum ewes there was an increase in the inhibitory effect of estradiol on LH release. Crowder and colleagues (1982) suggest that the failure of the ewe to release LH in response to estradiol is due to a decrease in the quantities of LH in the pituitary, rather than to a refractoriness to estradiol. This is consistent with the observation that the number of cytosolic receptors for estradiol in the pituitary remains constant after parturition (Crowder et al., 1981).

Elsaesser and Parvizi (1980) in the sow, found a small but significant increase in LH levels in sows treated with estradiol benzoate on day 35 but not on day 5 of lactation. Also Ramirez et al. (1985) found that treatment with estradiol induce more pronounced LH secretion in sows treated during the 3rd or 4th week of lactation compared to the 2nd week (Ramirez et al., 1985). Therefore these data suggest a gradual recovery of the neuroendocrine pathways mediating the stimulatory effects of estrogens with time from farrowing. Cox et al., (1988) gave lower doses of estradiol benzoate at three stages of lactation but did not observe negative or positive feed-back responses of LH. A low but chronic elevation in plasma estradiol level resulted in inhibition of endogenous LH secretion in sows (De Rensis et al., 1991). Moreover, the observation of an absence of estrus behaviour and ovulation, even in animals in which estrogen secretion is observed before weaning (Edwards and Foxcroft, 1983b), seems to indicate that a blockade of the positive estrogen feed-back mechanism may be a cause of lactational anovulation. Sesti et al. (1993) observed a biphasic response of LH secretion to estradiol during lactation, with an initial period of negative feed-back followed by a period of positive feed-back.

Finally, a study of the relationship between gonadotropin secretion and reproductive activity in the weaned sow (Shaw and Foxcroft, 1985) led to the proposal that the characteristics of LH secretion after weaning may depend on follicular development, and therefore, the extent of estradiol feed-back at the time of weaning. They suggested that in sows possessing ovarian follicles in a less advanced state there may be insufficient plasma estradiol to augment the weaning-associated rise in LH or, conversely, the hypothalamic-hypophysial axis of some sows 'escaped' the negative-feed-back effects of estrogen earlier then others.

The site at which estradiol affects LH release during lactation in sows is still not clear. Actions of estradiol during lactation have been described at both hypothalamic and pituitary levels (for review see Clarke, 1987; Nett, 1987). In sheep and pigs (Leakakos et al., 1987; De Rensis et al., 1991) estradiol treatment in late lactation induced chronic inhibition of LH secretion but this effect was eventually negated by the concomitant administration of LHRH. These data suggest that in the longer term estradiol acts mainly at the hypothalamic level to inhibit LH secretion, with an acute inhibitory effect at the pituitary level.

The neuroendocrine mechanisms by which estradiol decreases LH secretion at the hypothalamic level are also uncertain but opioids and dopamine may be involved. Evidence for a major opioid involvement in the block of LH secretion during lactation was reviewed by Haynes et al. (1989). Furthermore, it has been demonstrated that the effect of endogenous opioids on hypothalamic mechanisms controlling LH secretion are highly dependent upon the steroid milieu of the animal. The naloxone-induced elevation of LH is greatly attenuated, or totally absent, in gonadectomized rats but can be restored by pretreatment with estrogens (Bhanot et al., 1984). Moreover estrogen decreases the hypothalamic concentration of beta-endorphin and increases beta-endorphin release into the hypophyseal portal blood (Wardlaw at al., 1982; Sarkar and Yen, 1985). During lactation levels of opioids are increased by the suckling stimulus and therefore estradiol may interact with them in exercising an inhibitory effect on LH secretion.

Finally a link to neurotransmitter metabolism is suggested, since some estrogen metabolites (hydroxylated estrogen, catecholestrogens) compete with catecholamines for cathechol-O-methyl-transferase (COMt) (Brever et al., 1962). A higher affinity of COMt for catechol-estrogens could, for instance, result in the accumulation of norepinephrine and therefore, induce a similar effect to norepinephrine itself.

In conclusion, either a negative and a positive feed-back of estradiol-17beta on LH secretion could be observed during lactation. These effects may result from the type of treatment utilized and the period of lactation considered.

## 4. GONADOTROPIN SECRETION DURING GESTATION, PARTURITION AND LACTATION

Throughout the first part of gestation in the sow plasma LH concentrations have been reported to be relatively high and variable and can be compared with LH concentrations in the midluteal phase of the estrous cycle (Ziecik, et al., 1982, 1983). This pattern of LH secretion does not vary appreciably in late pregnancy although a decrease in pulse amplitude (Parvizi et al., 1976; Ziecik et al., 1982) and an increase in number of LH peaks (Kraeling et al., 1992) have been reported. During parturition plasma LH concentrations are very low, while FSH levels fall slightly (Ash and Heap 1975; Duggan et al., 1982).

In suckled beef cows the content of LH in the anterior pituitary increased from very low levels at parturition to levels similar to those present in acyclic animals by day 30 post-partum (Cermark et al., 1983; Moss et al., 1985). In ewes the pituitary content of LH gradually increased during the post-partum period, reaching levels similar to those observed in intact ewes between three and ten weeks post-partum (Jenkin et al., 1977; Moss et al., 1980, Crowder et al., 1982). Therefore, it is possible to conclude that in the ruminant, the lack of stores of LH in the anterior pituitary gland is one of the initial limitations on the resumption of normal estrous cycles.

In the pig low LH pituitary concentrations have been observed between the end of pregnancy and day 14 (Melampy et al., 1966) or even day 56 of lactation (Crighton et al., 1967) and these observations were initially interpreted as evidence for a block of the synthesis and release of LH at this time. However, as discussed above, the effect of GnRH injections has also been studied, with the aim of better identifying the ability of the pituitary to release LH during lactation. The concept of a releasable pool of LH was first described in the rat by Lu and colleagues (1976). They showed that the action of three consecutive injections of GnRH, administered at 50 minutes intervals, was markedly attenuated in lactating rats when compared with cyclic controls. In the sheep it has been observed that there is a releasable pool of LH during lactation and that the rise in pattern of LH secretion is independent of the dose of GnRH given (Kanchev et al., 1984). I has been suggested in the sow that during lactation the releasable amount of LH present in the pituitary changed (Stevenson et al., 1981b; see previous section), indicating that pituitary stores of releasable LH were lower on day 20 than day 10.

Generally, it has been reported that during lactation pituitary FSH levels are not depressed in the cow (Moss et al., 1985) or in the ewe (Moss et al., 1980; Cermark et al., 1983). During lactational anestrus in the sow pituitary levels of FSH concentrations return to normal soon after parturition (Crighton and Lamming, 1969) and these authors concluded that in the sow the suckling stimulus throughout lactation probably inhibited only the release of FSH, whilst inhibiting the synthesis and release of LH.

It is generally well accepted that mean plasma LH concentrations tend to increase gradually during lactation in many species. At least in cows, this results in a pulsatile pattern of gonadotropin release, with an LH pulse frequency of one every two hours, that is able to stimulate follicular growth and ovulation (Carruthers et al., 1980; Peters et al., 1981; Schallenberger et al., 1982b). Pulsatile LH activity appears to be absent, however, in the early stage of lactation in milked or suckling cows and Peters and colleagues (1981) and Riley and colleagues (1981) did not observe a well defined pulsatile pattern of LH until day 13 to 20 post-partum.

In contrast, in the pig, small fluctuations in LH secretion have been reported immediately after parturition (Kunavongrikt et al., 1983 b). However, a limited number of samples were taken each day in this work and it was not possible to describe the profile of LH secretion in detail. In a recent more detailed study, De Rensis et al. (1993b) describe active episodic-like secretion of LH in the first 36-48h after farrowing, irrespective of whether the sow was suckled or weaned after parturition. Depending on the extent of this post-partum secretory activity, suckling initially resulted in an inhibition of LH secretion between 55 and 72h post-partum. Once this inhibition has occurred, circulating LH levels remain generally suppressed during the first three weeks of lactation (Melampy et al., 1966; Parvizi et al., 1976; Edwards, 1982; Booman and Van de Wiel, 1980; Stevenson and Britt, 1980) and slightly increase in late lactation (Stevenson et al., 1981b). Sows showing an early return to estrus after weaning, have a well established pattern of episodic LH secretion prior to weaning (Shaw and Foxcroft, 1985; Foxcroft et al., 1987).

The patterns of plasma FSH secretion are much less clearly defined. This probably relates to difficulties in developing specific FSH radioimmunoassays. Although circulating concentrations of LH are lower in post-partum than in cyclic animals, concentrations of FSH are not suppressed (Walters et al., 1982b; Moss et al., 1985) or show little change (Lauderdale et al., 1965) during the post-partum period in cattle. A slight increase in plasma FSH throughout lactation has been reported in the sow (Stevenson et al., 1981) and Edwards and Foxcroft (1983) found that FSH concentrations tended to be higher at 5 weeks than at 3 weeks of lactation but did not consistently increase after weaning. The absence of any correlation between plasma FSH concentrations and time to return to estrus (Shaw and Foxcroft, 1985), or follicular development 48h after weaning (Foxcroft et al., 1987), supports the concept that lactational anestrus is not the result of a lack of FSH synthesis or release, thus reversing the conclusion drawn from earlier studies on pituitary FSH content.

## 5. PROLACTIN AND THE REGULATION OF PROLACTIN SECRETION

### 5.1 Origin and identity of prolactin

#### 5.1.1 Topography of prolacting-secreting cells.

Prolactin is synthesized not only by the lactotropes of the adenohypophysis but also by the endometrium (particularly the decidua) and the myometrium. PRL has also been localized by immunohistochemistry in a large variety of tissue and organs: mammary epithelium, liver, adrenal, granulosa cells, oocyte, proximal tubule of the kidney, endocrine pancreas, CNS, urethral gland and prostate (for review see Robyn and Meuris, 1988).

In the anterior pituitary, PRL is synthesized by the acidophilic lactotrope cells (Horrobin, 1977). For many years it was believed that growth hormone and PRL were secreted by separate and distinct pituitary cell types, the somatotropes and mammotropes (or lactotropes). However, recently, studies of Frawley and his group (for review see Frawley, 1989) indicate that many cells in normal pituitaries secrete both GH and PRL concurrently and these cells are defined as mammosomatotropes. Such mammosomatotropes are common in the pituitary of pregnant and lactating musk shrews, but are not abundant in those of virgin females (Ishibashi and Shiino, 1989). Therefore it has been suggested that mammasomatotropes impart "plasticity" to the pituitary gland by serving as transitional cells for the interconversion of classical mammotropes and somatotropes. In this manner, the long-term requirements of an animal for a particular hormone could be met without drastically changing the total number of cells within the pituitary gland. Estimates of the half life of PRL in cows range from 22 to 36 minutes ( Smith et al., 1977; Akers et al., 1980) and PRL appears to be bound and inactivated by the liver (Horrobin, 1977).

### 5.1.2 Molecular heterogeneity of PRL

Prolactin is a protein hormone of 198 amino acids and is a single chain formed into loops by three disulfide bonds. Considerable evidence has accumulated to suggest that human PRL is not a single molecule (Milmore and Ree, 1975). Although PRL is synthesized by only one gene on chromosome 6 in human, it is post-transcriptionally modified in various ways, resulting in different secreted molecular forms including a 199 amino-acid peptide, aggregates, glycosylated variants, cleaved forms producing 16k and 8k peptides, and PRL isohormones. Heterogeneity of PRL also has been reported in mouse (Sinha, 1980) and rat (Asawaroengchai et al., 1978). Three forms of human PRL have been described: little PRL (MW 22 kd) corresponding to the tomomomeric hormone; big PRL (MW 22kd), probably the dimeric form; and big-big PRL (apparent MW in excess of 160 kd) the structure of which remains unclear (Lewis, 1975; Niall, 1981; Fang and Refetoff, 1978: Garnier et al., 1978). Finally, the presence of 'medium-big' PRL (MW 80-90 kd) has also been reported which may be a tetramer of little PRL (Tanaka et al., 1984).

## 5.2 Spontaneous Prolactin secretion

When the mammalian pituitary gland is removed from hypothalamic influences by autotransplantion (Shin and Reifel, 1981), destruction of the mediobasal hypothalamus (Hall, 1984) or in vitro culture (Stewart et al., 1985), there is a prompt and sustained increase in PRL secretion that involves both de novo synthesis and increased release of the hormone. Thus, in contrast to the secretion of most other anterior pituitary hormones, the secretion of PRL is tonically inhibited by the hypothalamus. Spontaneous PRL secretion is not detectable in fish and birds since in these animals the secretion of PRL is mainly controlled by PRL releasing factor.

## 5.3 Autocrine and Paracrine control

Autocrine control of PRL secretion is the latest mode of hormone regulation to be demonstrated in the pituitary gland. Hagen et al. (1986) showed that there is an autocrine and paracrine stimulation of PRL secretion due to locally produced VIP. Moreover Nagy et al. (1988) suggest that the suppressive action of dopamine on PRL secretion must occur either by way of antagonizing the stimulatory effect of VIP or by inhibiting VIP secretion, or both. Denef and colleagues (1986) have extensively studied the paracrine interactions between pituitary cell types and described an interaction between gonadotropes and lactotropes. The agent mediating this effect appears to be angiotensin II, released from the gonadotropes in response to GnRH treatment. The physiologic significance of this paracrine regulation of PRL secretion by gonadotropes is unknown but it seems to be involved in chronic changes in PRL secretion such as those occurring after estrogen treatment.

## 5.4 Hypothalamic Control

The mechanisms controlling PRL synthesis and secretion are not simple. Increased PRL secretion above low basal levels in various physiological states appears to be mediated by both a block of the secretion of inhibitory factor and by the release of a stimulatory factor. The areas of the central nervous system causing both stimulation and inhibition of PRL release have been especially well studied in the ewe and seem to be located in the anterior and posterior medio-basal hypothalamus (Wolinska et al., 1977).

During lactation a number of exteroceptive stimuli play an important role in maintaining the elevation of the circulating PRL (Jakubowski and Terkel, 1985). It has been demonstrated in a number of species that the frequent application of the suckling/milking stimulus is one of the most crucial. In fact the suckling stimulus induces a prompt and marked release of PRL that exceeds that shown in other conditions, such as at the time of the preovulatory surge of LH, in the early stages of pregnancy and in response to stressful stimuli and food intake.

The neuro-anatomical mechanisms that induce PRL secretion during lactation have been extensively studied but are not yet clearly understood. Various neurotransmitters are involved in the hypothalamic regulation of PRL secretion, but the role of these neurotransmitters is poorly understood because of the complexity of the neuroanatomic connections involved. However, using pharmacological agents that either potentiate or antagonize the action of such neurotransmitters, their potential involvement in the regulation of PRL has been demonstrated. Thus norepinephrine (Voogt and Carr, 1974; Lawson and Gala, 1975), serotonin (Kamberi et al., 1971) and histamine (Pontiroli and Pozza, 1978) have been described as being involved in the mechanism regulating PRL secretion.

The identification of the neuro-hormonal substances which transmit the signal from the midbrain to the hypothalamus and then that regulate PRL release during lactation has been the object of extensive studies. More than 30 neuroactive substances have been tested for PRF activity and among these thyrotropin-releasing hormone (TRH), oxytocin, angiotensin II and bradykinin have been considered as potential candidates.

### 5.4.1 Prolactin Inhibitory Factors (PIF)

In 1963 Talwalker et al. demonstrated that a factor extracted from the hypothalamus was able to inhibit the release of PRL; from that moment, numerous studies have sought to identify this factor(s). The observation that the hypothalamus contains high concentrations of catecholamines and that the axons of some hypothalamic neurones rich in catecholamines terminate in the external layer of the median eminence, in close apposition to the primary portal capillaries (Fuke and Hokfelt, 1966), suggested that dopamine exerts an inhibitory effect on PRL secretion. These conclusions were confirmed by evidence that the administration of a variety of dopaminergic agonists and antagonists results in a decrease or increase of PRL release, respectively (Weiner et al., 1978; Smith and Wagner 1985). Dopamine reaches the anterior pituitary via two-vascular routes: the long portal vessels from the hypothalamus and the short portal vessels from the posterior pituitary. There is evidence indicating that dopamine from the posterior pituitary contributes significantly to the suppression of PRL release under a variety of endocrine conditions (Peters et al., 1981). There are also data in the rat showing that hypothalamic dopamine turnover is reduced in lactation (Selmanoff and Wise, 1981; Mena et al., 1976; Demarest et al., 1983) and the concentration of dopamine in the hypophysial portal vessels is acutely reduced in response to suckling (Plotsky and Neill, 1982).

With regard to lactational anestrus, it is not clear if there is a PRL induced increase in hypothalamic dopamine turnover and, if present, whether this mechanism is involved in the suppression of GnRH secretion. However it seems that during lactation there is a change in the properties of the hypothalamic tuberoinfundibular dopamine neurones since they become unresponsive to the stimulatory actions of PRL (Demarest et al., 1983).

The mechanisms by which dopamine acts to suppress PRL secretion are not firmly established. It was found that dopamine can hyperpolarize the plasma membrane, reduce spontaneous action potentials and then reduce calcium fluxes leading to reduced intracellular [Ca2+] and to the attenuation of calcium mobilization by TRH (Schofield, 1983; Sherey et al., 1986).

In 1979, Kebabian and Calne introduced the concept of two different dopamine receptors: D1 receptors that stimulate adenylate cyclase activity of the target cells and the D2 receptor that, when stimulated, does not increase cAMP synthesis. It seems that PRL release is primarily governed by the tonic inhibitory influence of dopamine acting on D2 class receptors (MacLeod et al., 197). The activation of those receptors by PRL induces an attenuation of the stimulatory effect of TRH on phosphoinositide metabolism in the lactotropes and therefore induces a block of the synthesis and secretion of PRL (MacLeod et al., 1988). However in a recent paper (Curlewis et al., 1993) it was shown that injection of the dopamine D1 agonist SKF 38393 increased PRL. Furthermore utilizing hypothalamo-pituitary disconnected sheep they demonstrated that these drugs do not act directly at the pituitary gland secretion. By the application of the reverse haemolytic plaque assay, it has been shown that there are two subpopulations of lactotropes with differential responsiveness to dopamine; one population with a high and one with a low PRL secretion rate. Dopamine preferentially inhibited PRL secretion from cells with elevated secretory rates (Louque et al., 1988). Furthermore the data of Winiger et al. (1987) revealed subclasses of lactotropes with distinct intracellular Ca2+ response characteristics. A substantial subfraction of the lactotropes, but not all, respond to dopamine with a rise in intracellular Ca2+. These observations demonstrate that there is a heterogeneity of the lactotrope population in primary cultures of pituitary cells. It has been suggested that this response-type heterogeneity is a general feature of cells whose activities are controlled by external factors, since it allows an organ like the pituitary to adapt to a particular physiological status, (i.e. pregnancy, lactation) by alterations in the relative proportions of lactotrope populations without the need to alter drastically its composition of major cell types.

There are reports of a reduction in dopamine concentrations in the median eminence (Moyer et al., 1979) and anterior pituitary (Demarest et al., 1983) during suckling, as well as a decrease in tuberoinfundibular dopamine activity (Selmanoff and Wise, 1981). However, there are also studies that did not identify these changes (Voogt and Carr, 1974) during lactation. De Greef and Visser (1981) observed that electrical stimulation of the mammary nerve to simulate suckling, evokes a marked increase in plasma PRL levels but only a transient fall in the levels of DA in portal blood. Moreover, although an abrupt withdrawal of dopamine induces a rise in PRL (Plotsky and Neill, 1982), the brevity of the reduction in DA appears insufficient to fully account for the massive rise in PRL during suckling. Ben-Jonathan et al. (1980) showed that dopamine is important for the rapid decline in PRL immediately after removal of the pups and for maintaining low PRL levels during non suckling intervals. Therefore from this data it is possible to summarize that the control of PRL secretion during lactation is due in part to a brief removal of dopamine inhibition and in part to the stimulation of PRF and/or increased lactotropes responsiveness to the action of PRF.

It has been suggested that gamma aminobutyric acid (GABA) has also PIF activity (Schally et al., 1977; Lux Lantos et al., 1993) and that the gonadotropin-releasing hormone associated peptide (GAP) is able to inhibit the release of PRL (Nikolics et al., 1985). However if GAP, released at the same time as GnRH, was a potent inhibitor of PRL release, it is difficult to understand how synchronous pulses of both PRL and LH occur, i.e., during sleep in puberty and the preovulatory LH surge in the estrous and menstrual cycle.

### 5.4.2 Prolactin Releasing Factors (PRF)

Besides the well characterized hypothalamic inhibitory regulation of PRL secretion, a good deal of evidence exists for a stimulatory mechanism. However, much less is known about the chemical identity, localization and regulation of PRL releasing factors (PRF).

TRH was one of the first peptides shown to stimulate PRL release by a direct action on the pituitary (Tashjian et al., 1971). Despite this well established action, considerable disagreement exists regarding its role as a physiologic PRF, as several physiologic states exist in which TRH and PRL are not released together. In the rat Jacobs et al. (1978) suggest that TRH has a stimulatory effect. However while the suckling stimulus induced a fifty-fold rise in plasma PRL, it was either ineffective (Riskind et al., 1984) or caused only a small and delayed increase (Blake, 1974) in plasma TSH. Furthermore, immunoneutralization of TRH blocks the rise in TSH, but not in PRL in response to electrical stimulation of the hypothalamus. Moreover, injection of TRH antiserum decreases basal TSH release but does not abolish the PRL response to suckling (Sheward et al., 1985). These data and the frequent dissociation between the release of TSH and PRL, therefore make it difficult to exactly describe the role of TRH in the control of PRL.

Vasoactive Intestinal Peptide (VIP) and the peptide histidine isoleucine can also stimulate PRL release in vitro (Samson et al., 1980) and in vivo (for review see Kaji et al., 1985). In particular VIP is deeply involved in the autocrine and paracrine stimulation of PRL secretion (see chapter III). Finally, Samson et al. (1984) demonstrated that secretin could also be involved in the regulation of PRL secretion.

The administration of oxytocin and arginine vasopressin to lactating rats deprived of their endogenous peptide by posterior pituitary lobectomy stimulated milk ejection and restored water balance, but failed to affect plasma PRL levels (Murai and Ben-Jonathan, 1987). Samson and colleagues (1986) observed that plasma oxytocin rose significantly just before the PRL surge that occurred in response to suckling in lactating rats. The infusion of a specific anti-oxytocin serum immediately before pup reinstatement in lactating females, delayed and significantly reduced PRL release compared to controls. It therefore seems possible to conclude that oxytocin plays a physiological role in the control of PRL secretion during lactation at least in the rat.

Regarding the sow, Tyson (1977) reported that severance of the nerve supply to the nipple, as well as nipple anaesthesia, abolished nursing-induced PRL release, but not milk ejection, suggesting a dissociation of the mechanisms controlling oxytocin and PRL release. Angiotensin II and bradykinin are also involved in the secretion of PRL but they are not the major contributors to PRL release.

Traditionally, endocrinologists have held the view that the anterior and posterior lobes of the pituitary are not functionally related. In contrast, detailed studies in vivo of directional blood flow through the pituitary have shown that the anterior lobe can receive blood from the adjacent posterior lobe (Page, 1983). It has also been reported that the posterior pituitary contains a small peptide that plays an important role during lactation and that this peptide is different from other well known peptides known to be PRL stimulating factors. Ben-Jonathan et al. (1988) observed that posterior pituitary lobectomy in lactating rats abolished the suckling-induced rise in PRL as compared with the 40-fold increase in sham operated controls; they were also able to isolate an acid extract from the posterior pituitary with a potent stimulatory effect on PRL cells. However, this peptide has not yet been isolated and therefore more studies are required to prove the existence of this PRF. In a detailed study Hyde et al. (1987) have eliminated several well-known peptides such as oxytocin, angiotensin II, TRH and VIP as being the stimulating factor. Therefore it seems possible that the posterior pituitary contains a small peptide distinct from known PRL secretagogues that plays an important role during lactation in PRL secretion. The involvement of a serotonergic mechanism is supported by pharmacological manipulations. Depletion of serotonin by p-chlorophenylalanine, a synthesis inhibitor, blocks the sucking induced rise in PRL and this is reversed by repletion with the serotonin precursor 5HTP (Kordon et al., 1973).

Data regarding the post-partum period indicate that the noradrenergic (noradrenaline and adrenaline) pathway does not appear to be involved in the suckling-induced release of PRL. The synthesis of hypothalamic noradrenaline does not change following suckling (Voogt and Carr, 1974) and the suckling-induced release of PRL is not affected by blockade of noradrenaline and adrenaline synthesis (Carr et al., 1977).

The opioids are also able to increase the secretion of PRL in several reproductive periods (Meites, 1988; Grandson and Guidotti, 1977; Rivier et al., 1977; Reid et al., 1981; Tolis et al., 1975; Crowley, 1988). Virtually all the opioid peptides that have been tested stimulate PRL release (for review see Crowley, 1988) and the effect can be antagonized by naloxone or naltrexone. However there are discordances regarding the opioid receptors involved in the control of PRL secretion. Akil et al. (1984) and Zuckin et al. (1981) report evidence for an existence of mu, delta and kappa receptors. In contrast, Spiegel et al. (1982) and Koening and Krulich (1984) described that the beta-endorphin effect on PRL secretion is not blocked by a mu1-antagonist, suggesting that this site is not involved in mediating the stimulatory effect of this peptide on PRL. Furthermore, Koening et al. (1984) and Laedem et al. (1987) report that delta receptors are not involved in the control of PRL secretion.

During lactation the blockade of opiate receptors with naloxone results in a decrease of PRL secretion in the rat (Sirinathsinghji and Martini, 1984), sow (Mattioli et al.,1986), sheep and cow (for review see Haynes et al., 1989) but apparently not in women (Grossman et al., 1981; Delitala et al., 1981; Cholst et al., 1984). In both the rat and sow, there was also a significant increase in LH secretion. Gordon et al., (1987) found an increase in beta-endorphin levels in the hypothalamo-pituitary portal blood in response to the suckling stimulus and a close correlation with peripheral PRL concentrations and bet-endorphin pulses in the pituitary gland. Also, in lactating sows, there are reports of an effect of naloxone injection on PRL plasma levels. In sows treated at day 15 (Mattioli et al., 1986), at day 21 (Armstrong et al., 1988b) and day 22 (Barb et al., 1986a) post-partum, naloxone induced a significant decrease in plasma PRL. The infusion of naloxone or temporal removal of the litter was able to lower PRL concentrations but, although PRL levels remained depressed in naloxone treated sows they increased to pretreatment values in sows resuming suckling. The authors suggest that these results may be due to acute tolerance to naloxone following an infusion regimen, as has been reported in other species (Ebling and Lincoln, 1985).

The mechanism(s) by which opioids control PRL secretion during lactation is not well understood. Suggestions are for an effect on tuberoinfundibular dopamine neurons and, more precisely, an inhibitory effect on dopamine secretion. Armstrong et al. (1988b) observed that 0.5 mg\kg of naloxone is sufficient to suppress serum PRL for sixty minutes in the sow. In a second experiment treatment with morphine after weaning at day 25 induced a depression of PRL secretion. This observation was unexpected and the authors propose that previous exposure of the sow to suckling and, by inference, endogenous opioids, in conjunction with the high dose of morphine used, caused down regulation or desensitization of opiate receptors.

However, Arita and Porter (1984) infused DA intravenously to rats pretreated with alfa-methyl tyrosine and established that the suppression of dopamine activity alone may not account fully for the PRL-releasing effects of opioids. Therefore opioids may also promote the secretion of PRL releasing factors, although the type of PRL releasing factors increased by opioids has been not well established. In fact TSH secretion (and presumably TRH release) is decreased by opioids (Van vugt et al., 1980) and there are no data of opioid effects on the release of VIP. Instead there are data that endogenous opioids activate the central serotoninergic system that in turn stimulates PRL secretion (Spampinato et al., 1979; Koening et al., 1980a; Demarest et al., 1981).

In conclusion, the hypothalamic control of PRL release induced by suckling depends on an inhibitory action on dopamine release and the stimulatory effect on PRL releasing factors of the endogenous opioids.

### 5.5 Short loop feed-back

A short loop feed-back operates in which PRL itself can alter the release of dopamine. High concentrations of PRL increase dopamine output from the hypothalamus, which suppresses further release of PRL from the pituitary and vice versa if PRL levels are low. This mechanism was first demonstrated in the rat (Hokfelt and Fuxe, 1972) and has now been demonstrated in the mouse and hamster (Steger et al., 1985; Bartke et al., 1987) and suggested in primates (Herbet and Martenz, 1983). PRL could reach the hypothalamus from the peripheral circulation or, alternatively, by way of retrograde blood flow in the portal vessels (Mezey et al., 1982).

### 5.6 Steroid hormones

In mammals estrogen stimulates PRL secretion with actions both at the hypothalamic level to reduce dopamine release and at the pituitary level to affect dopamine action (McNeilly, 1987). Estrogen causes a significant increase in both plasma and pituitary concentrations of PRL and in PRL mRNA levels within the pituitary (Stanley et al., 1986). An extra effect of estrogen is to increase the number of binding sites for the stimulatory substances for PRL secretion, such as TRH, in vivo (Kraeling and Rampacek, 1986; Hall, 1984) and in pituitary cells in vitro (Hall, 1984). Bookfor et al. (1986) demonstrated that estradiol acts directly on cultures of male pituitary cells to modify the proportions of cells that release growth hormone, PRL or both hormones, which indicates that estradiol may convert cells that release only growth hormone (somatotropes) to those that release both growth hormone and PRL (mammosomatotropes).

Considerable evidence has been accumulated suggesting that the metabolism of various gonadal hormones are important for the manifestation of hormonal activity. Regarding PRL, Rodrigueaz-Sierra and Balke (1982) have observed that catecholestrogens are able to elevate serum PRL levels on a chronic basis. Finally, progesterone has been reported to act within the CNS and directly on the lactotropes (Fink, 1988a) to regulate the PRL secretion.

## 6. INTERRELATIONSHIP BETWEEN PROLACTIN AND GONADOTROPIN SECRETION

The origin of the involvement of PRL in the mechanisms regulating reproduction can only be speculated. This might derive from the primitive association of PRL with osmoregulation, since most euryhaline fish species enter fresh water to rear their young, and tropical fish species are stimulated to produce their broods by an influx of freshwater (Meites, 1988). The "eft" drive in amphibia is also reported to be PRL dependent.

Therefore, the association between PRL and reproduction in higher mammals might be an outgrowth of that stimulation. During the estrous cycle in the sow there are two fairly distinct peaks in plasma PRL: one occurs four days before estrus and the other during the sexual receptivity phase (Brinkley et al., 1973; Dusza and Krzymoska, 1979). A major elevation in PRL also occurs around parturition (Van Landeghem and Van de Wiel, 1978; Dusza and Krzymowska, 1981).

PRL plays many biological roles in the post-partum period. It has a significant role in the establishment of maternal behaviour (Bridges et al., 1987) and increases gene expression of milk proteins (Rosen et al., 1980). It also plays a major role, together with estrogen and progesterone, in the homing process of lymphocytes originating from Peyer patches of the maternal gut. These lymphocytes produce IgA antibodies excreted in milk as secretory IgA specific to antigens present in the gut of the mother (Weitz-Carrington et al.,1978). Apart from these important functions during lactation, PRL also has an important role in the mechanism regulating the return of sexual function after farrowing.

### Human

It has been reported that in women PRL secretion by the pituitary gland is rapidly increased during late gestation due to the effect of high levels of placental estrogen (Kletzky et al., 1980). After labour, maternal plasma PRL drops at a very slow rate and, in women as in other species, the post-partum levels of PRL are related to the amount of suckling stimulus and then to the length of lactational anestrus.

The close association between the plasma levels of PRL and the duration of infertility has led to the suggestion that PRL per se may be involved in the suppression of gonadotropin secretion in women. Evidence that PRL can directly influence LH secretion and affect the resumption of ovarian activity came from the observation in the human that in some pathological conditions, such as pituitary microadenoma resulting in hyperprolactinaemia, the levels of FSH and LH are low. However, if the pathologically high concentration of PRL was suppressed by treatment with bromocriptine, normal menstrual cycles resumed (Rolland et al., 1975). However, since the suckling-related increase in PRL is not acutely related to any inhibition of basal or pulsatile secretion of LH (Glasier et al., 1984), the decline in PRL may only reflect the decrease in suckling activity and may not have a direct effect on the resumption of follicular development.

Nevertheless, during lactation, here is also evidence for inhibitory effects of PRL at the ovarian level. Increasing amounts of PRL inhibit progesterone secretion in human and porcine granulosa cells in vitro (McNatty, 1974; Veldhuis et al., 1980b). Furthermore, McNeilly et al. (1982) postulated that high levels of PRL inhibit follicular steroidogenesis not only by interfering with aromatase activity but also by reducing the production by the theca of the androgen precursors necessary for estrogen production.

### Rat

In the rat the plasma concentrations of PRL during lactation are strictly correlated with the suckling stimulus and enhanced by increasing litter size (Coppings et al., 1979). Female rats that are suckled continuously have significantly higher PRL levels than those nursing four times daily (Tucker et al., 1988) and the fall in PRL secretion towards the end of lactation is presumably a result of decreased suckling frequency (Ford and Melampy, 1973). A fall in PRL during the latter stages of lactation has been observed but it is not clear if this is due to the reduction in the suckling intensity or due to other factors.

In order to investigate the possibility that the limited PRL response to suckling in late lactation resulted from decreased suckling intensity provided by older pups, Mathji et al. (1979) and Selmanoff and Wise (1981) used cross-fostering to replace older pups with younger neonates at the end of lactation. They found that the response of PRL to suckling was markedly blunted compared with the response profile at the beginning of lactation. Moreover Selmanoff and Selmanoff (1983) observed that in rats, the suckling-induced release of PRL at day 20 post-partum had a slower onset, markedly reduced peak height and a faster return to baseline than the response at day 10. Therefore these data seem to confirm that during late lactation the PRL release mechanism becomes less responsive to the suckling stimulus.

In an attempt to ascertain the relative contributions of the suckling stimulus and PRL to the inhibition of gonadotropin secretion, the post-castration rises of LH and FSH during lactation in female rats have been investigated. Results from these studies suggest that during early lactation the suckling stimulus contributes more than PRL to the suppression of LH, while during the latter stages of lactation PRL may account for the decrease in the post-castration rises in gonadotropin secretion (Smith, 1978). However, the mechanisms involved in the suppression of basal gonadotropin secretion during lactation may be different from the mechanisms involved in the suppression of the post-castrational response.

When suckling is maintained in the absence of elevated PRL, rats showed an early resumption of estrous cycles (Lu et al., 1976) and since the injection of bromocriptine during lactation resulted in a significant increase in LH and FSH and an earlier resumption of estrous cycles (Lu, 1983), the high PRL levels rather than the suckling stimulus may be responsible for the suppression of gonadotropins. However, in these as in many others studies, suckling intensity was not controlled. This may explain the contrary findings of Van der Schoot and co-workers (1982), who demonstrated that injection of bromocriptine into lactating rats produced no changes in the gonadotropin secretion even though PRL was reduced.

It is not yet clear if during lactation there is a PRL induced increase in hypothalamic dopamine turnover (short loop feed-back) and, if present, whether this mechanism is involved in the suppression of GnRH secretion. Although it has been reported that PRL release induced by stress or suckling increases dopamine turnover, as shown by indirect turnover studies (Mena et al., 1976), but there is evidence that in the rat the hypothalamic dopamine turnover is reduced in lactation (Selmanoff and Wise, 1981; Demarest et al., 1983) and the concentration of dopamine in the hypophysial portal vessels is acutely reduced in response to suckling (Plotsky and Neill, 1982). Furthermore, Demarest et al., (1983) observed that during lactation in the rat there is a change in the properties of the hypothalamic tuberoinfundibular dopamine neurones and they become unresponsive to the stimulatory actions of PRL.

### Cow

As in other species, lactation in the cow is associated with high levels of PRL (Webb and Lamming, 1981) and the increased PRL concentrations are strictly correlated with the presence of the suckling stimulus. In fact calf removal was associated with a rapid decline in the plasma PRL associated with an increase in basal LH secretion (Wright et al., 1987). In contrast to the rat, plasma PRL concentrations are similar in suckled and milked cows (Carrunthers and Hafs, 1980) and in cows suckling one or two calves (Wheeler et al., 1982) and no correlation has been found between PRL concentrations and the length of the anestrous period in either milked or suckled cows (Webb and Lamming, 1981). Since suppression of plasma PRL with bromocriptine does not affect the time to onset of estrus (for review see Montgomery, 1982), and infusion of PRL failed to affect the pattern of LH secretion (Williams and Ray, 1980; Forrest et al., 1980), it therefore seems that PRL does not play a key role in the lactational anestrus in this species.

### Sheep

In the sheep the plasma concentration of PRL is low during the breeding season, increases in response to increasing day length, and is high during the period of lactational anestrus (Walton et al., 1977). As in other species the PRL response to suckling in the sheep declines throughout lactation McNeilly (1972).

A number of studies have been performed in sheep to distinguish the differential effects of suckling and high plasma PRL levels on lactational anestrus. For example, it has been revealed that the duration of lactational anestrus is dependent on the concentration of circulating PRL (Kann and Martinet, 1975; Kann et al., 1977). In fact treatment with bromocriptine to suppress PRL, or severance of the neural pathway for the suckling stimulus, which abolishes the suckling-induced PRL surge whilst maintaining basal PRL throughout lactation, are followed by an early restoration of ovarian activity. This may suggest that the suckling-induced PRL surges are responsible for prolonging the anestrous period through suppression of gonadotropin secretion. In contrast, Fitzgerald and Cunningham, (1981) report that treatment of lactating ewes with bromocriptine may not advance the onset of estrus post-partum and, furthermore, this treatment does not appear to influence the basal or pulsatile release of LH in lactating ewes, even though the release of LH in response to exogenous GnRH may be increased (Wright et al., 1981a).

In conclusion it is not yet clear if PRL has a direct role in establishing lactational anestrus in the sheep and further studies are necessary to clarify the effect of bromocriptine treatment on gonadotropin release.

### Sow

In the sow extremely high concentrations of PRL have been detected on the day before farrowing (Dusza and Krzymowska, 1981; Van Landeghem and Van de Wiel, 1978). This increase is an essential prerequisite to a normal lactation. After parturition, plasma PRL gradually decreases to the still relatively elevated concentrations associated with lactation (Bevers et al., 1978; Stevenson et al., 1981).

In the early post-partum period therefore there is already an active secretion of PRL, with mean PRL concentrations ranging between 12.0 and 89.6 ng/ml (De Rensis et al., 1991). Since high variability was observed in plasma PRL between animals was observed in this study and these differences were not due to seasonal changes or to differences in litter size, it was suggested that the observed variability was due to genetic differences between animals. The functional significance of this considerable variability in PRL secretion in suckling sows needs to be established in future studies. However, from a biological viewpoint it is interesting to note that among the suckled sows studied, the lowest concentrations of plasma PRL were detected in a sow which nevertheless farrowed 13 live piglets and from which 13 pigs were eventually weaned. During mid-lactation plasma PRL concentrations remain high (Threlfall et al., 1974; Bevers et al., 1978; Van Landeghem and Van de Wiel, 1978; Dusza and Krzymowska, 1981) and then decline between day 20 and day 30 post-partum (Stevenson et al., 1981).

The PRL response to the suckling stimulus is characterized by a rapid increase and then a return to the baseline levels after 30 to 60 minutes (Van Landeghem and Van de Wiel, 1978). The separation of the piglets from the mother for periods of four hours or more resulted in a rapid decline in plasma PRL and in increases in basal LH secretion. In the sow, the administration of exogenous PRL, from immediately after weaning until the occurrence of estrus, did not influence plasma LH concentrations, nor the time of estrus and the preovulatory LH surge occurred in all experimental animals (Dusza et al., 1990). However, the dose of PRL used (0.125 mg) was in our opinion low compared to the usual concentrations of PRL observed during lactation. In contrast, Van de Wiel et al. (1985) significantly lowered plasma LH, compared with untreated animals, by the infusion of purified porcine PRL in the sow for 24 hours immediately after weaning. Furthermore, whilst the administration of exogenous PRL during the separation of piglets did not prevent the increase in LH levels, the extent of this increase was significantly reduced in sows infused with PRL compared with those which were not (Booman and Van de Wiel, 1980). In conclusion, it appears that in the sow the suppression of LH during lactation is partly caused by hyperprolactinaemia. However, PRL alone may not mediate differences in sow fertility, as in sows with a delayed return to estrus after weaning, plasma PRL concentrations decrease in the same way as in animals with a normal return to estrus (Benjaminsen, 1981; Edwards and Foxcroft, 1983; Van de Wiel et al., 1985). It has also been reported that the progressive ability of the pituitary to discharge LH as the interval from parturition increases, was not associated with modifications in PRL concentrations (Bevers et al., 1981). Nevertheless in individual sows, high circulating PRL may be associated with reduced ovarian development in the presence of adequate gonadotropin stimulation (Foxcroft et al., 1987).

In contrast to findings in the rat, in which suckling may also have the effect of stimulating PRL synthesis, in the sow, Threlfall et al. (1974) reported a decreased content of pituitary PRL in suckled as compared to non suckled sows; these finding may indicate that the suckling stimulus in the pig leads to a higher rate of PRL release than synthesis, whilst the reverse is true for the rat.

Another approach to the study of PRL during lactation has been the examination of the effect of the PRL inhibitor, bromocriptine, on LH release. While Mattioli and Seren (1985) did not find any differences in LH after bromocriptine treatment, Kraeling (1982) observed that treatment with bromocriptine was associated with significantly lower serum

LH concentrations in treated animals compared to control. Similarly, Bevers and colleagues (1983) found that the reduction of PRL by bromocriptine was associated with increased mean LH levels. If PRL is decreased by removal of the piglets, this does not enhance pituitary sensitivity to GnRH stimulation (Bevers et al., 1981), but if PRL levels are decreased by bromocriptine, the injection of GnRH induced a smaller increase in plasma LH levels in treated lactating sows compared with non-treated animals (Mattioli and Seren, 1985).

## 7. PROLACTIN INFLUENCE ON THE OVARY

A direct inhibitory action of PRL on the ovaries has been demonstrated in the pig; in vitro incubation of granulosa cells from small follicles with PRL resulted in a decrease in progesterone secretion (Veldhuis et al., 1980). Similarly, results have been reported in earlier studies with human granulosa cells in which increasing amounts of PRL added to the culture medium inhibited the secretion of progesterone in a dose dependent manner (McNatty et al., 1974). It has also been demonstrated that high levels of PRL can block FSH induction of aromatase in the estrogen synthetase system (Wang et al., 1980; Dorrington and Gore-Langton, 1981). Such inhibitory effects of PRL are consistent with in vivo evidence. For example, the induction of elevated PRL by repeated injection of TRH in ewes, resulted in a suppression of estradiol secretion by the action of PRL on the ovary (McNeilly and Baird, 1983).

Numerous studies, both in vivo and in vitro, provide support for a direct inhibitory effect of PRL on follicular secretion of estradiol-17beta. Studies in the rat (for review see Dusza, 1990) clearly showed that PRL inhibits estradiol-17beta secretion by granulosa cells under all experimental conditions. It has also been observed that high concentrations of prolactin suppressed ovarian estradiol-17b and estrone secretion in pigs. Dusza et al., (1990) reported that PRL administration for 60 h during the follicular phase of the estrous cycle in intact gilts decreased the concentrations of estradiol-17beta in peripheral plasma on the 2nd and 3rd day of PRL treatment.

Little is known about the role of PRL in the growth and development of ovarian follicles. Wang et al. (1980) suggested that a marked inhibitory action on estrogen secretion by the granulosa cells could result in the termination of follicular growth and the initiation of atresia in some follicles. However, the action of PRL on follicular development is not yet well understood. In the cow, for example, the injection of bromocriptine or the infusion with PRL antiserum during the luteal phase of the estrous cycle does not affect peripheral progesterone concentrations or the length of estrous cycle (Hoffman et al., 1974). Furthermore, PRL receptors have not been identified on bovine thecal and granulosa cells (Bevers et al., 1985), whereas in the pig the number of prolactin receptors on granulosa cells decreases with increasing size of follicles (Rolland and Hammond, 1975).

In conclusion, while there is evidence for a requirement for prolactin during the estrous cycle in maintaining growth and/or development of follicles, the mechanisms are poorly understood. Studies in which blood levels of PRL have been suppressed pharmacologically with bromocriptine, ergocornine or with antiserum to PRL (for review see McNeilly, 1982) suggest that only minimal amounts of PRL are needed for normal follicular growth. With regard to the lactational period in pigs, the relationship between the high plasma PRL levels and the block of follicular development needs further investigation but it seems probable that there is potential for a local inhibitory effect of PRL on the ovary.

## 8. CONCLUSION

In conclusion, from the studies reported above, a schematic representations can be used to explain the stimulatory effects of the suckling stimulus on PRL secretion and the involvement of these mechanisms in the control of reproductive inactivity during the post-partum period (see Fig. I.1 and 1.2).

Neural inputs from the nipples could, directly or indirectly (for example by the opioidergic system), inhibit the secretion of PIFs and increase the PRFs, thus increasing the secretion of PRL. The release of the opiate beta-endorphin due to inputs from the nipple is thought to suppress (directly or indirectly, for example by the dopaminergic system) the discharge of GnRH. As a consequence of this decrease, GnRH "self-priming" is reduced, which decreases the releasable pool of LH in the pituitary and the frequency and magnitude of episodic LH release. It is important to consider that the influence of various neurotransmitters on the regulation of PRL secretion may differ between species. For example, during lactation the blockade of opiate receptors with naloxone results in a decrease of PRL secretion in the rat (Sirinathsinghji and Martini, 1984) and sow (Mattioli et al., 1986), but apparently not in women (Cholst et al., 1982). In both the rat and sow there is also a significant increase in LH secretion.

Consequently, conclusions made by extrapolating data based on one species may be misleading when considering the role of PRL in lactational anestrus in other species. It is also important to remember the complicating effects of progesterone secretion from corpora lutea that are maintained during lactation in the rat but not in other species.

## TABLE 1.1 EFFECT OF ADMINISTRATION OF NALOXONE ON LH SECRETION IN DOMESTIC SPECIES.

| Species | Reproductive period | Naloxone effect | Reference |
|---|---|---|---|
| Sheep | 1. Lamb 12 weeks old | LH increase | Ebling et al., 1989 |
| | 2. Lamb 12 weeks old, ovx+E17 implants | LH increase | |
| | 1. Early post-natal | LH increase | Finnie et al., 1989 |
| | 2. Late prepubertal | LH increase | |
| | 3. Prepubertal | No effect | |
| | 1. Prepubertal lamb, 20, 23, 25, 30 weeks old | LH increase | Matthews and Murdoch, 1984<br>Churchill and Rawlings, 1987 |
| | 1. Yearling ewes, seas. anestrus | LH increase | Brooks et al., 1986b |
| | 1. Adult seasonally anestrus | No effect | Brooks et al., 1986b |
| | 1. Ovx lamb 3 weeks old, after removing E2 implants | LH increase | Ebling et al., 1989 |
| | 1. Prepubertal feed-restricted lambs | LH attenuated response | Foster et al., 1988 |
| | 1. Cyclic luteal phase | LH freq. decr.<br>LH ampl. incr. | Whisnant and Goodman, 1988 |
| | 2. Cyclic follicular phase | LH freq. incr.<br>LH ampl. decr. | |
| | 3. Ovx ewe + E2 | LH ampl. incr. | |
| | 4. Ovx ewe + P4 | LH freq. incr.<br>LH ampl. incr. | |
| | 1. Luteal phase | LH freq. incr. | Yang et al., 1985 |
| | 2. Follicular phase | LH freq. incr. | Brooks et al., 1985<br>Brooks et al., 1986 |
| | 1. Follicular phase | LH freq. incr.<br>LH ampl. decr. | Currie and Rawlings, 1987 |
| | 1. Suckling | LH increase | Newton et al., 1988 |
| | 2. None suckling | LH increase | |
| | 1. Ram breeding season | LH increase | Ebling and Lincoln, 1985 |
| | 2. Ram anestrous | No effect | |
| | 1. Ovx ram + testosterone, breeding season | No effect | Lincoln et al., 1987 |

| | | | |
|---|---|---|---|
| | 1. Suckling 10, 14, 18, 22, 26 days post-partum | LH increase | Gregg et al., 1986 |
| | 2. No suckling 10 days post-partum | LH increase | |
| | 1. 12 weeks old | Limited increase | Ebling et al., 1987 |
| | 2. 20 weeks old, ovx + E2 shortly before puberty | LH increase | |
| | 3. 35 weeks old ovx + E2<br>E2 replaced with P4 | No effect<br>No effect | |
| | 1. Luteal phase | LH increase | Brooks et al., 1986b<br>Currie and Rawlings. 1987<br>Trout and Malven, 1987 |
| | 1. Ovx breeding season | LH increase | Brooks et al., 1986c |
| | 2. Ovx seasonally anestrus | LH increase | |
| | 3. Breeding season + E2 | No effect | |
| | 4. Seasonally anestrus + E2 | No effect | |
| | 1. Seasonally anestrus | No effect | Meyer and Goodman, 1986<br>Brooks et al., 1986b<br>Yang et al., 1988 |
| | 1. Ovx | Limited effect | Brooks et al., 1986 |
| | 2. Ovx + P4 | LH increase | |
| | 3. None breeding season | | |
| | - ovx | No effect | |
| | - ovx + E2 | No effect | |
| | - ovx + E2 + P4 | LH increase | |
| | 1. Ovx + P4 | LH increase | Trout et al., 1987 |
| | - After P4 removal | No effect | |
| | - Single E2 injection | LH increase | |
| | 1. Ovx, breeding season | LH increase | Cermak and Nett, 1985 |
| | 2. Ovx, none breeding season | LH increase | |
| | 3. Ovx, breeding season + E2 | Greater LH increase | |
| | 4. None breeding season | Greater LH increase | |
| Cow | 1. Late follicular phase | LH ampl. incr. | Short et al., 1987 |

| | | | |
|---|---|---|---|
| | 2. Luteal phase | No effect | |
| | 1. Early post-natal | LH increase | McDonald et al., 1990 |
| | 2. Late prepubertal | LH increase | |
| | 3. End of infantile period | No effect | |
| | 1. None suckled, post-partum | No effect | Whisnant et al., 1986a |
| | 2. Suckled day 40, post-partum | LH increase | |
| | 1. Calf removal, day 48 post-partum | No effect | Whisnant et al., 1986c |
| | 1. Suckled day 18 and 19 post-partum | LH increase | Cross et al., 1987 |
| | 1. Suckled day 14, 28, 42 | LH increase | Whisnant et al., 1986a<br>Whisnant et al., 1986b |
| | 2. After weaning | No effect | |
| | 3. None suckled | No effect | |
| | 1. Follicular phase | LH increase | Short et al., 1987 |
| | 2. Luteal phase | No effect | |
| | 1. Suckled day 18-19 | LH increase | Cross et al., 1987 |
| | 2. Weaned day 21 | No effect | |
| Horse | 1. Diestrous | LH increase | Sharp et al., 1985 |
| | 2. Estrous | No effect | |
| | 3. Ovx | No effect | |
| | 1. None breeding season | No effect | Sharp et al., 1985 |
| Pig | 1. Very young immature male pig | FSH increase,<br>no LH increase | Trudeau et al., 1988<br>Trudeau et al., 1989 |
| | 1. 10 week old males | No FSH incr.,<br>LH suppressed | Prunier et al., 1989 |
| | 1. Luteal phase | LH increase | Barb et al., 1985 |
| | 2. Early follicular phase | No effect | Barb et al., 1986a |
| | 3. Late follicular phase | No effect | |
| | 1. 40 h after weaning | No effect | Barb et al., 1986b |
| | 1. Prepubertal gilts | No LH increase | Barb et al., 1988 |
| | 2. Ovx prepubertal gilts + P4 | No LH increase | |

| | | |
|---|---|---|
| 3. Ovx gilts + P4 | LH increase | |
| 4. Ovx gilts + E2 or E2 + P4 | LH increase | |
| 5. Adult gilts + P4 at ovx | LH increase | |
| 1. Prepubertal gilts, feed-restricted | LH suppressed | Cosgrove et al., 1990 |
| 2. Prepubertal gilts, re-fed | LH suppressed | |
| 1. Suckled | LH increase | Mattioli et al., 1986 |
| 1. Suckled | LH increase | Barb et al., 1986b |
| 1. Suckled | LH freq. incr. | Armstrong et al., 1988b |

ABBREVIATION USED
ovx = ovariectomized
E2 = estradiol-17beta
P4 = progesterone
ses. = seasonal

**Fig. 1.1** Possible mechanisms involved in the neuroendocrine control of gonadotropin and PRL secretion during lactation.

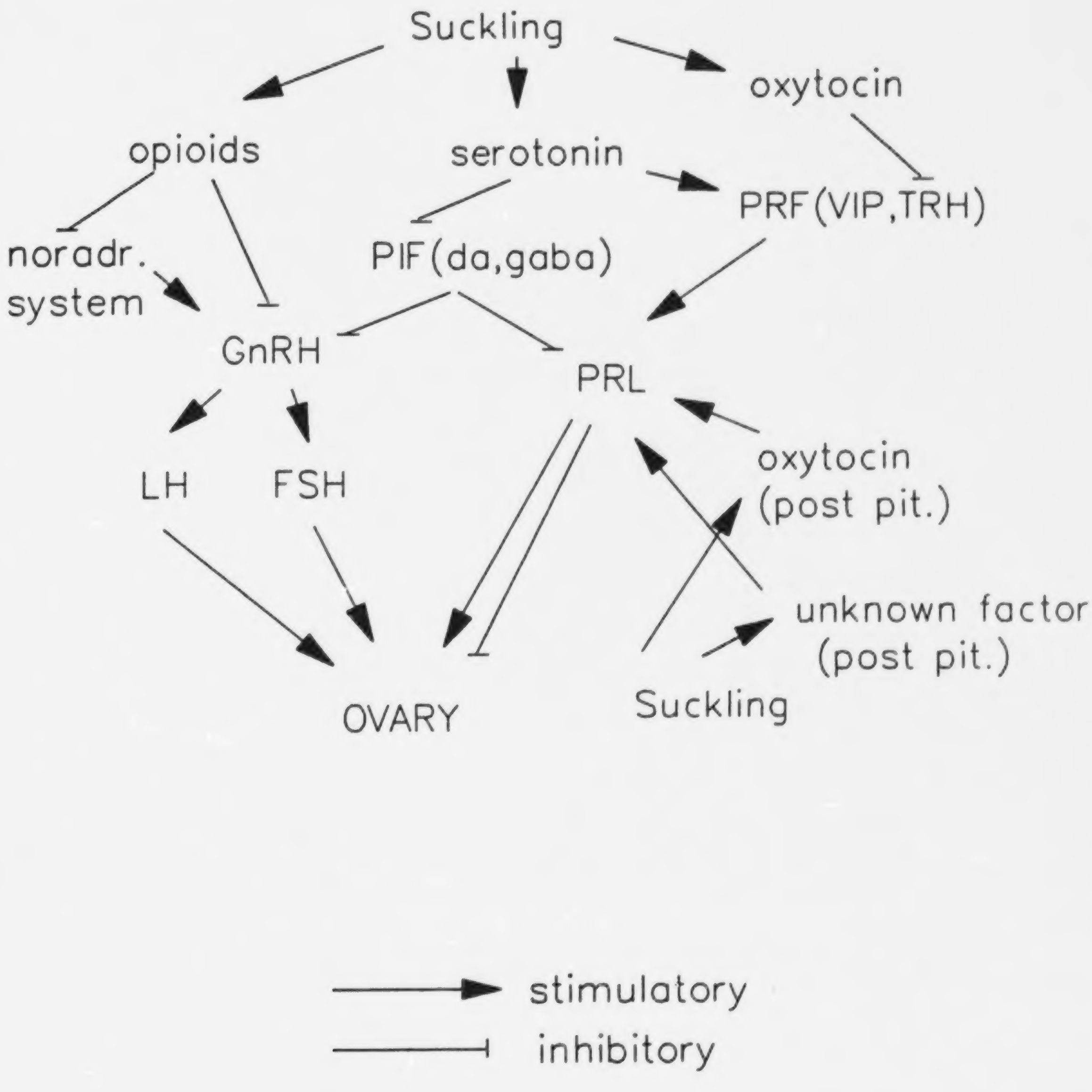
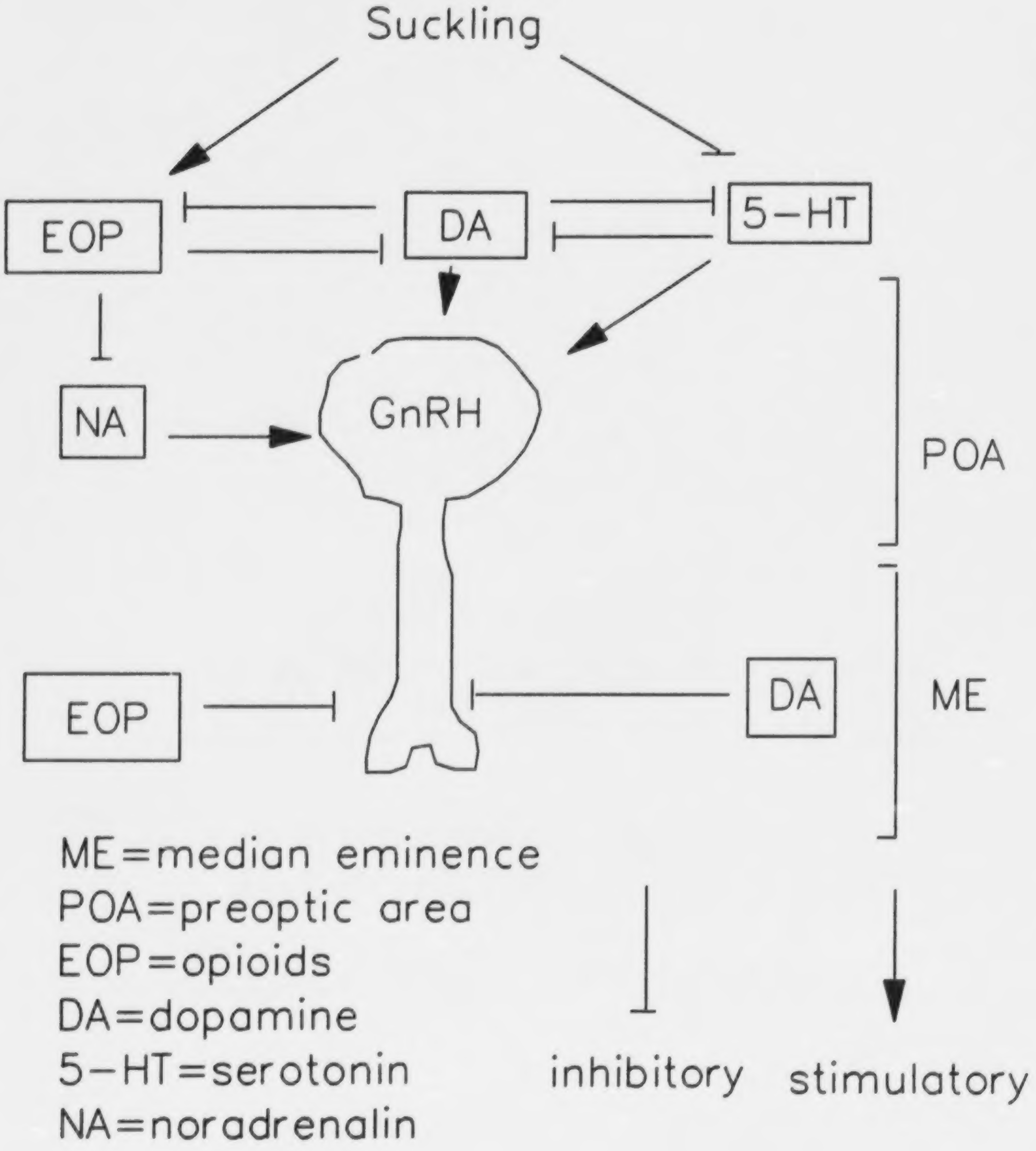

Suckling
oxytocin
opioids
serotonin
PRF(VIP,TRH)
noradr.
system
PIF(da,gaba)
GnRH
PRL
LH
FSH
oxytocin
(post pit.)
unknown factor
(post pit.)
Suckling
OVARY
stimulatory
inhibitory

**Fig. 1.2** Schematic diagram of the suggested interactions between the dopaminergic and opioidergic system and the control of GnRH\LH secretion. Plus and minus signs indicate stimulatory and inhibitory effects, respectively. This diagram is highly simplified and does not take account of the many other potential neuroendocrine regulators of GnRH release.


Suckling
EOP
DA
5-HT
NA
GnRH
POA
EOP
DA
ME
ME=median eminence
POA=preoptic area
EOP=opioids
DA=dopamine
5-HT=serotonin
NA=noradrenalin
inhibitory
stimulatory

## 9. BIBLIOGRAPHY

Ackland, J.F., Schwartz, N.B., Mayo, K.E., Dodson, R.E. (1992). Nonsteroidal signals originating in the gonads. Physiological Reviews, 72:731-786.

Adams, T.E., Spies, H. G. (1981). Binding characteristics of gonadotropin-releasing hormone receptors throughout the estrous cycle of the hamster. Endocrinology., 108:2245-2248.

Akers, R.M., Goodman, G.T. & Tucker, H.A. (1980). Clearance and secretion rates of prolactin in dairy cattle in various physiological states. Proc. Soc. exp. Biol. Med., 164:115-119.

Akil, H., Warson, S.J., Young, E., Lewis, M.E., Khachaturian, H., Walker, J.M. (1984). Endogenous opioids, biology and function. Ann. Rev. Neurosci., 7:223.

Ahern, K., Fuxe, K., Hamberger, L., Hokfelt, T. (1978). Turnover changes in the tubero-infundibular dopamine neurons during the ovarian cycle of the rat. Endocrinology., 88:1915-1924.

Alder, B.E., Crowley, W. R. (1984). Modulation of LH release and catecholamine activity by opiates in the female rat. Neuroendocrinol. 38:248-253.

Arita J, Porter JC (1984). Relationship between dopamine release into hypophysial portal blood and prolactin release after morphine treatment in rats. Neuroendocrinol. 38:853-857.

Armstrong, D.J., Kraeling, R.R., Britt, J.H. (1988). Effects of naloxone or transient weaning on secretion of LH and prolactin in lactating sow. J. Reprod. Fertil., 83:301-308.

Armstrong, J.D., Kraeling, R.R., Britt, J.H. (1986). Effect of bolus administration of naloxone on secretion of LH and prolactin in lactating sows. J. Anim. Sci., (Suppl. 1) Abstract 322.

Armstrong, J.D., Kraeling, R.R., Britt, J.H. (1988a). Morphine suppresses luteinizing hormone concentrations in transiently weaned sows and delays onset of estrus after weaning. J. Anim. Sci., 66:2216-2222.

Armstrong, J.D., Kraeling, R.R., Britt, J.H. (1988b) Effects of naloxone or transient weaning on secretion of LH and prolactin in lactating sows. J. Reprod. Fertil., 83:301-308.

Asawarroengchai, H., Russel, S.M., Nicoll, C.S. (1978). Electrophoretically separable forms of rat prolactin with different bioassay and radioimmunoassay activities. Endocrinology., 102:407-414.

Ash, R.W., Banks, P., Bailes, G., Broad, S., Heap, R.B. (1973). Plasma oestrogen, progesterone and corticosteroid concentration in the pregnant, parturient and lactating sow. J. Reprod. Fertil., 33:359-360.

Ash, R.W., Heap, R.B. (1975). Oestrogen, progesterone and corticosteroid concentrations in peripheral plasma of sows during pregnancy, parturition, lactation and after weaning. J. Endocrinol., 64:141-154.

Autelitano, D.J., Clements, J.A., Nikolaidis, I., Canny, B.J., Funder, J.W. (1987). Concomitant dopaminergic and glucocorticoid control of pituitary proopiomelancortin messenger ribonucleic acid and beta-endorphin levels. Endocrinology., 121:1689-1693.

Ayer, M.S., Fink, G., Grieg, F. (1974a). Changes in the sensitivity of the pituitary gland to luteinizing hormone releasing factor during the estrus cycle in the rat. J. Endocrinol., 60:47-64.

Ayer, M.S., Fink, G. (1974). The role of sex steroid hormones in modulating the responsiveness of the anterior pituitary gland to luteinizing hormone releasing factor in female rat. J. Endocrinol., 62:553-572.

Baird, D.T., Swanston, I.A., Scaramuzzi, R.J. (1976). Pulsatile release of LH and secretion of ovarian steroids in sheep during the luteal phase of the oestrous cycle. Endocrinology., 98, 6:1490-1496.

Baldwin, D.M., Stabenfeldt, G.H. (1975). Endocrine changes in the pig during late pregnancy, parturition and lactation. Biol. Reprod., 12, 4:508-515.

Barb, C.R., Kraeling, R.R., Rampacek, G.B., Whisnant, C.S. (1986). Opioid inhibition of luteinizing hormone secretion in the post-partum lactating sow. Biol. Reprod., 35:368-371.

Barb, C.R., Kraeling, R.R., Rampacek, G.B., Leshin, L.S. (1987). Opioid modulation of follicle stimulating hormone (FSH) and prolactin (PRL) secretion in post-partum sow. In: Advances in Experimental Medicine and Biology. Ed. by Mahesh, V.B., Dhindsa, D.S., Anderson, E., Karla, S.P. Plenum, New York, 219:647-652.

Barb, C.R., Estienne, M.J., Kraeling, R.R., Marple, D.N., Rampacek, G.B., Rahe, C.H., Sarti, J.L. (1990). Endocrine changes in sows exposed to elevated ambient temperature during lactation. Domest. Anim. Endocrinol., 8, 1:117-127.

Barb, C.R., Barret, J.B., Wright, J.T., Kraeling, R.R., Rampacek, G.B. (1990). Opioid modulation of lH secretion by pig pituitary cells in vitro. J. Reprod. Fertil., 90:213-218.

Barb, C.R., Kraeling, R.R., Rampacek, G.B. (1991). Opioid modulation of gonadotropin and prolactin secretion in domestic farm animals. Dom. Anim. Endocrinol., 91:15-27.

Barraclough, C.A., Wise, P.M. (1982). The role of catecholamines in the regulation of pituitary LH and FSH secretion. Endocrine rev., 3:91-119.

Beck, T.W., Convey, E.M. (1974). Luteinizing hormone after estradiol and GnRH in spayed heifers. J. Anim. Sci., 39:198 (abstr.) 237.

Beck, T.W., Smith, V.G., Seguin, B.E., Convey, E. M. (1976). Bovine serum LH, GH and PRL following chronic implantation of ovarian steroids and subsequent ovariectomy. J. Anim. Sci., 42:461-468.

Beck, W., Engelbart, S., Gelato, M., Wuttke, W. (1977). Antigonadal effect of prolactin in adult castrated and in immature female rats. Acta. Endocrinol., 84:62-71.

Ben-Jonathan, N., Arbogast, L.A., Hyde, J.F. (1989). Neuroendocrine regulation of prolactin release. Progress in Neurobiology, 33:399-447.

Benjaminsen, E. (1981). Plasma prolactin in the sow with emphasis on variation in resumption of ovarian activity after weaning. Acta Vet. Scad., 22:67-77.

Besser, G.M., Parkes, L., Edwards, C.R.W., Forsyth, I.A., McNeilly, A.S. (1972). Galatorrhea: successful treatment with reduction of plasma prolactin levels by bromoergocryptine. Br. Med. J., 3:669-671.

Bevers, M.M., Willemse, A.H., Kruip, T.A.M. (1978). Plasma prolactin levels in the sow during lactation and post weaning period as measured by radioimmunoassay. Biol. Reprod., 19:628-634.

Bevers, M.M., Willemse, M.H., Kruip, T.A.M., Van de Wiel D.F.M. (1981). Prolactin levels and the LH response to synthetic LHRH in lactating sow. Anim. Reprod. Sci., 4:155-163.

Bevers, M.M., Willemse, M.H., Kruip, T.A.M. (1983). The effect bromocriptine on luteinizing hormone levels in the lactating sow: evidence for a suppressive action by prolactin and the suckling stimulus. Acta Endocrinol., 104: 261-265.

Bevers. M., Dieleman, T.H., Kruip, T.A.M., Willemse, A.H. (1985). Follicular development in the heifers chronically treated withe Bromocriptine. In: Follicular growth and ovulation rate in farm animals. Ed. by J.F. Roche and D.O'Callaghan. Martinus Nijhoff Publishers, pp. 45-53.

Bhanot, R., Wilkinson, M. (1984). The inhibitory effects of opiates on gonadotropin secretion is dependent upon gonadal steroids. J. Endocrinol., 102:133-141.

Bicknell, R.J. (1985). Endogenous opioid peptides and hypothalamic neuroendocrine neurones. J. Endocrinol., 107, 437-446.

Blake, C.A., (1974) Stimulation of pituitary prolactin and TSH release in lactating and proestrus rats. Endocrinology., 94, 503-508.

Blank, M.S., Panerai, A.E., Friesen, H.G. (1979). Opioid peptides modulate LH secretion during sexual maturation. Science 203:1129-1131.

Blank, M.S., Roberts, D.L. (1982). Antagonist of GnRH blocks naloxone induced elevation in serum LH. Neuroendocrinol., 5:309-312.

Blank, M.S., Fabbri, A., Catt, K.J., Dufau, M.L. (1986). Inhibition of luteinizing hormone release by morphine and endogenous opiates in cultured pituitary cells. Endocrinology., 118:2097-2101.

Boesgaard, S., Hagen, C., Hangaard, J., Andersen, A.N., Eldrup, E. (1991). Pulsatile gonadotrophin secretion and basal PRL levels during dopamine D1 receptor stimulation in normal women. Fert. and Ster., 55:281-286.

Bookfor, F.R., Hoeffler, J.P., Frawley, S.L. (1986). Estradiol induces a shift in cultured cells that release prolactin or growth hormone. Am. J. Phys., 250 (Endocrinol. Metab. 13):E103-E105.

Booman, P., Van De Wiel, D.F.M. (1980). Lactational anoestrus in the pig: possible relationship with hyperprolactinaemia. Report B-157 Instituut voor veeteeltkundig Onderzoek "Schoonoord", Driebergseweg 10 Zeist, Holland.

Bowers, G.Y., Currie, B.L., Johansson, K.N.G., Folkers, K. (1973). Biological evidence that separate hypothalamic hormones release the follicle stimulating and luteinizing hormones. Biochem. Biophys. Res. Commun., 50:20-26.

Brever, H., Vogel, W., Knuppen, R. (1962). Enzymatische methylierung von 2-Hydroxy-oestradiol-(17β) durch eine S Adenosyl Methionine acceptor - O - methyltransferase der raltenleber hoppe seyler's 2. Physiol. Chem., 327, 217-222.

Bridges, R.S., Loundes, D.D., Dibiase, R., Tate Ostroff, B.A. (1987). In: Prolactin: basic and clinical correlates. Ed. by Macleod, R.M., Thorner, M. and Scapagnini, U., Fidia Research Series, Liviana Press, Padova, 1:591-599.

Brinkley, H.J., Wilfinger, W.W., Young, E.P. (1973). Plasma prolactin in the estrous cycle of the pig. J. Anim. Sci., 37, 303 (Abstr.).

Britt, J.H., Armstrong, J.D., Moore, K.L., Sesti, L.A.C. (1993). Involvement of opioids in regulation of LH secretion during lactational- or nutritional-induced anestrus in pig and cattle. In: Opioids in farm animals. Ed. by N.Parvizi, pp. 34-54.

Britt, J.H., Armstrong, J.D., Cox, N.M., Esbenshade, K. (1985). Control of follicular development during and after lactation in the sow. J. Reprod. Fertil., 33:37-54

Britt, J.H., Kittok, R.J., Harrison, D.S. (1974). Ovulation, oestrus and endocrine response after GnRH in early post partum cows. J. Anim. Sci., 39:915-919.

Britt, J.H., Armstrong, J.D., Moore, K.L., Sesti, L.A.C (1993). Involvement of opioids in regulation of LH secretion during lactational- or nutritional-induced anestrus in pig and cattle.In: Opioids in farm animals, EDS N.Parvizi, pp. 34-54.

Brooks, A.N., Lamming, G.E. and Haynes, N.B. (1986a). Endogenous opioid peptides and the control of gonadotrophin secretion. Res. in Vet. Sci. 41:285-299.

Brooks, A.N., Lamming, G.E., Lees, P.D., Haynes, N.B. (1986b). Opioid modulation of LH in ewes. J. Reprod. Fertil., 76:693-708.

Brooks, A.N., Haynes, N.B. Yang, K-P. and Lamming, G.E. (1986c). Ovarian steroid involvement in endogenous opioid modulation of LH secretion in seasonally anoestrous mature ewes. J. Reprod. Fertil., 76:709-715.

Brown-Grant, K., Greig, F. (1975) A comparison of changes in the peripheral plasma concentrations of luteinizing hormone and follicle stimulating hormone in the rat. J. Endocrinol., 65:389-397.

Carpenter, L.S., Anderson, L.L. (1985). Pulsatile infusion of LHRH: effects on LH hormone secretion and ovarian function in prepubertal gilts. Anim. Reprod. Sci., 9:261-272.

Carr, L., Conway, P., Voogt, J. (1977). Role of norephinephrine in the release of prolactin induced by suckling and estrogen. Brain Res., 133:305-314.

Carruthers, T.D., Hafs, H.D. (1980a). Suckling and four times daily milking influence on ovulation,oestrus and serum LH, glucocorticoid and PRL in post partum Holstein. J. Anim. Sci., 50:919-925.

Carruthers, T.D., Convey, E.M., Kesner, J.S., Hafs, H.D., Cheng K.W. (1980b). The hypothalamo-pituitary -gonadotropic axis of suckled and non suckled dairy cows post partum. J. Anim. Sci., 51:949-956.

Carter, M.L., Dierschke, D.J., Putledge, J.J., Hanser, E.R. (1980). Effect of GnRH and calf removal on pituitary ovarian function and reproductive performance in post partum beef cows. J. Anim. Sci., 51:903-910.

Casper, R.F., Alpin-Rubillovitz, S., (1985). Progestins increase endogenous opioid peptide activity in post-menopausal women. J. Clin. Endocr. Metab., 60:34-36.

Cermark, D.L., Braden, T., Manils, J., Niswender, G.D., Nett, T.M. (1983). Contents of hypothalamic GnRH, pituitary FSH and LH, and pituitary receptors for GnRH and estradiol in post partum suckled beef cows. J. Anim. Sci., 57 (Suppl 1) (abstract 322).

Cetel, N.S., Olvigley, M.E., Yen, S.S.C (1985). Naloxone induced PRL secretion women, evidence against a direct PRL stimulatory effect of endogenous opioids. J. Clin. Endocr. Metab. 60:191-196.

Chao, C.C., Malven, P.V., Moss G.E. (1986). Direct opioid regulation of pituitary release of bovine luteinizing hormone. Life Sci., 39:527-534.

Chappel., S.C., Barraclough, C.A. (1976). Hypothalamic regulation of pituitary FSH secretion. Endocrinology., 98:927-935.

Chiappa, S.A., Fink, G. (1977). Releasing factor and hormonal changes in the hypothalamic-pituitary-gonadotrophin and -adenocorticotrophin system before and after birth and puberty in male, female and androgenized female rats. J. Endocrinol., 72:211-224.

Ching, M., (1983). Morphine suppresses the proestrous surges of GnRH in pituitary portal plasma of rats. Endocrinology., 112, 2209-2211.

Cholst, I.N., Wardlaw, S.L., Newman, C.B., Frantz, A. (1984). Prolactin response to breast stimulation in lactating women is not mediated by endogenous opioids. Am. J. Obstet. Gynecol. 150: 558-561.

Churchill, I.J., and Rawlings, N.C.,1987. Naloxone enhances episodic luteinizing hormone and suppress episodic follicle stimulating hormone secretion in prepubertal ewe lambs. Biol. Reprod. 36: (suppl.1) abstr. 418.

Cicero, T.J., Bordger, T.M., Willcox, C.E., Bell, R.D., Meyer, E.R. (1977). Morphine decreases luteinizing hormone by an action on the hypothalamic - pituitary axis. Journal of Pharmacology and Experimental Therapeutics, 203:548-555.

Cicero, T.J., Owens, D.P., Schmoeker, P.F., Meyer, E.R. (1983). Morphine induced enhancement of the effect of naloxone on serum luteinizing hormone levels in male rat: specificity for mu antagonists. J. Pharmacol. exp. Ther., 226:770-774.

Clarke, I.J., Wright, P.J., Chamley, W.A., Burman, K. (1984). Differences in the reproductive endocrine status of ewes in early post partum period and during seasonal anoestrus. J. Reprod. Fertil., 70:591-597.

Clarke, I.J, Cummins, J.T. (1982). The temporal relationships between gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) secretion in ovariectomized ewes. Endocrinology., 111:1737-1739.

Clarke, I.J., Cummins, J.T., De Krestser, D.M. (1983). Pituitary gland function after disconnection from direct hypothalamic influences in the sheep. Neuroendocrinol., 36:376-384.

Clarke, I.J., Cummins, J.T., Crowder, M.E., Nett, T.M. (1984) Pituitary receptors for gonadotrophin-releasing hormone in relation to changes in pituitary and plasma LH in ovariectomized-hypothalamo pituitary disconnected ewes. 1 Effect of changing frequency of gonadotrophin-releasing hormone pulses. Biol. Reprod. 37:749-754.

Clarke, I.J. (1987). Control of GnRH secretion. J. Reprod. Fertil., Suppl., 34:1-8.

Coppings, R.J., McCann, S.M. (1979) Stimulatory feed-back action of estradiol in intact and ovariectomized lactating rats. Biol. Reprod., 21:219-227.

Cosgrove. J.R., Booth, P.J., Foxcroft, G.R., (1991). Opioidergic control of gonadotrophin secretion in the prepubertal gilt during restricted feeding and realimentation. J. Reprod. Fertil., 91:277-284.

Cox, N.M., Britt, J.H. (1982a). Relationship between endogenous gonadotropin-releasing-hormone, gonadotropins, and follicular development after weaning in sows. Biology Reprod., 27:70-78.

Cox, N.M., Britt, J.H. (1982b). Pulsatile administration of gonadotropin releasing hormone to lactating sows: endocrine changes associated with induction of fertile estrus. Biology Reprod., 27:1126-1137.

Cox, N.M., Ramirez, J.L., Matamoros, I.A., Bennet, W.A. (1988). Estrogen induces estrus unaccompanied by a preovulatory surge of luteinizing hormone in suckled sows. Biol. Reprod. 38:592-599.

Creese, I., Sibley, D.R., Hamblin, M.W., Leff, S. (1983) The classification of dopamine receptor: relationship to radioligand binding. Ann. Rev. Neuroscience 6:43-71.

Crighton, D.B. & Lamming, G.E. (1969). The lactational anoestrus of sows: the status of the anterior pituitary-ovarian system during lactation and after weaning. J. Endocrinol., 93:507-519.

Crighton, D.B. (1967). Effects of lactation on the pituitary gonadotrophins of the sow. In: Reproduction in the Female Mammal. Ed. by G.E. Lamming and E.C. Amoroso. London:Butterworth pp. 223-238.

Cross, J.C., Rutter, L.M., Manns, J.C. (1987). Effect of progesterone and weaning on LH and FSH responses to naloxone in post-partum beef cows. Dom. Anim. Endocrinol., 4:111-112.

Crowder, M.E., Gilles, P.A., Tamanini, C., Moss, G.E., Nett, T.M. (1982). Pituitary content of gonadotropin and GnRH-receptors in pregnant, post partum and steroid treated ovariectomized ewes. J. Anim. Sci., 54:1235-1242.

Crowder, M.E., Gilles, P.A., Tamanini, C. (1981). Changes in hypophysid receptors for GnRH and estradiol in steroid treated and post-partum ewes. Biol. Reprod., 24:(Suppl. 1), 121A.

Crowley, W.R. (1988). Role of endogenous opioid neuropeptides in the physiological regulation of luteinizing hormone and prolactin secretion. In: Peptide hormones: effects and mechanisms of action. Eds, A. Negro-Villar, P.M. Conn. 3:79-166.

Curlewis, J.D., Clarke, I.J., McNeilly, A.S. (1993). Dopamine D1 receptor analogues act centrally to stimulate prolactin secretion in ewes. J. Endocrinol., 137:457-464

Currie, W.D. and Rawlings, N.C. (1987). Naloxone enhances LH but not FSH release during various phases of the estrous cycle in the ewe. Life Sci., 41:1207-1214.

Currie, W.D. and Rawlings, N.C. (1989). Fluctuation of responsiveness of LH and lack of responsiveness of FSH to prolonged infusion of morphine and naloxone in the ewe. J. Reprod. Fertil., 86:359-366.

Currie, W.D., Ravindra, J., Kingsbury, D.L., Rawlings, N.C. (1992). Independence of progesterone blockade of the LH surge in ewes from opioid activity at naloxone-sensitive receptors. J. Reprod. Fertil., 95:249-255.

Dailey, R.A., Deave, D.R., Goodman, R.L. (1987). Neurotransmitter regulation of LH and PRL secretion. J. Reprod. Fertil., Suppl. 34:17-26.

Deaver, D.R., Dailey, R.A. (1982). Effects of dopamine, norepinephrine and serotonin on plasma concentrations of luteinizing hormone and prolactin in ovariectomized and anestrous ewe. Biol. Reprod. 27:624-632.

Deaver, D.R., Dailey, R.A. (1983). Effects of dopamine and serotonin on concentrations of luteinizing hormone and estradiol-17beta in plasma of cycling ewes Biol. Reprod. 28:870-877.

Deaver, D.R., Keisler, D.H., Dailey, R.A. (1987). Effects of domperidone and thyrotropin-releasing hormone on secretion of LH and PRL during the luteal phase and following induction of luteal regression in sheep. Dom. Anim. Endocrinol. 4:94-102

Dearry, A., Gingrich, J.A., Falardeau, P., Fremeau, R.T., Bates, M.D., Caron, M.G. (1990). Molecular cloning and expression of the gene for human D1 dopamine receptor. Nature, 347: 72-76.

Debeljuk, L., Arimura, A., Schally, A.V. (1972). Effect of estradiol and progesterone on the LH release induced by LHRH in intact diestrous rats and anestrous ewes. Proc. Soc. exp. Biol. Med., 139:774-777.

De Greef, W.J., Plotshky, P.M., Neill, J.D., (1981). dopamine levels in hypophysial stalk plasma and prolactin levels in peripheral plasma of the lactating ret:effect of a stimulated suckling stimulus. Neuroendocrinol., 32:229-233.

DeLeew, R., Van't Veer, C., Goos, H.Jr, Van Oordt, P.G. (1988). The dopaminergic regulation of gonadotrophin-releasing hormone receptor binding in the pituitary of the African Catfish, Clarias gariepinus. Gen. Comp. Endocrinol., 72:408-415.

Delitala, G., Devilla, L., Arata, L. (1981). Opiate receptors and anterior pituitary hormone secretion in man. Effect of naloxone infusion. Acta Endocrinol., 97:150-154.

Del Pozo, E., Varga, L., Wyss, H., Tolis, G., Froiesen, H., Wenner, R., Vetter, L., Uttewiler, A. (1974). Clinical and hormonal response to bromocriptine (CB 154) in the galttorhea syndromes. J. Clin. Endocrinol. Metab., 39:18-21.

Demarest, K.T., McKay, D.W., Riegle, G.D., Moore, K.E. (1983). Biochemical indices of tuberoinfundibular dopaminergic neuronal activity during lactation: a lack of response to prolactin. Neuroendocrinol., 32:24-27.

Demarest, K.T., Moore, K.E. (1981). Disruption of 5-hydroxytryptaminergic neuronal function blocks the action of morphine on tuberoinfundibular dopaminergic neurons. Life Sci. 28:1345-1347.

Denef, C., Baes, M., Schramme, C. (1986). Paracrine interactions in the anterior pituitary: Role in the regulation of prolactin and growth hormone secretion. In: Frontiers in Neuroendocrinol., Ed. by W.F. Ganmong and L. Martini. Raven Press, New York. pp. 9-155.

De Rensis, F., Hunter, M.G., Grant, S.A., Lancaster, R.T., Foxcroft, G.R. (1991). Effect of estrogen administration on endogenous and luteinizing-hormone-releasing-hormone-induced luteinizing hormone secretion and follicular development in the lactating sow. Biol. Reprod. 44:975-982.

De Rensis, F., Foxcroft, G.R. (1993a). Endogenous opioid modulation of gonadotropin and prolactin secretion in the post-partum ewe. In: Opioids in farm animals, Ed. N.Parvizi, Landwirtschaftsverlag GmbH, 4400 Münster-Hiltrup, Germany. pp. 15-33.

De Rensis, F., Hunter, M.G., Foxcroft, G.R. (1993b). Suckling-induced inhibition of LH secretion and follicular development in the early post-partum sow. Biol. Reprod, 48:964-969.

De Rensis, F., Cosgrove, J.R., Foxcroft, G.R. (1993c). Luteinizing hormone and prolactin responses to naloxone vary with stage of lactation in the sow. Biol. of Reprod, 48:970-976.

Dhariwal, A.P.S., Nallar, R.,Batt, M., McCann, S.M. (1967). Separation of follicle-stimulating hormone-releasing factor from luteinizing hormone-releasing factor. Endocrinology., 76:290-294.

Dierschke, D.H., Bhattacharya, A. N., Atkinson, L.E., Knobil, E. (1970). Circhoral oscillations of plasma LH levels in the ovariectomized rhesus monkey. Endocrinology., 87:850-853.

Diekman, M.A., Malven, P. (1973). Effect of ovariectomy and estradiol on LH patterns in ewe. J. Anim. Sci., 37:562.

Diez-Guerra, F.J., Augood, S., Emson, P.C., Dyer, R.G. (1986). Morphine inhibits electrically stimulated noradrenaline release from slices of rat medial preoptic area. Neuroendocrinol., 43:89-91.

Domanski, E., Przekop, F., Polkowska, J. (1980). Hypothalamic centres involved in the control of gonadotrophin secretion. J. Reprod. Fertil., 58:493-499.

Domanski, E., Przekp, F., Skubiszewski, B., Wolinska, E. (1975). The effect and site of action of indoleamines on the hypothalamic centers involved in the control of LH release and ovulation in the sheep. Neuroendocrinol., 17:265-273.

Donnelly, P.J. and Dailey, R.A. (1991). Effect of dopamine norepineprine and serotonin on secretion of luteinizing hormone, follicle stimulating hormone and prolactin in ovariectomized, pituitary stalk-transected ewes. Dom. Anim. Endocrinol., 8(1):87-98.

Dorrington, J. and Gore-Langton, R.E. (1981). Prolactin inhibits oestrogen synthesis in the ovary. Nature, Lond., 290:600-602.

Drouin, J., Lagace, L,. Labrie, F. (1976). Estradiol-induced increase of the LH responsiveness to LH releasing hormone (LHRH) in rat anterior pituitary cells in culture. Endocrinology., 99: 1477-1480.

Drouva, S. V., Gallo, R.V. (1977). Further evidence for inhibition of episodic LH release in adult ovariectomized rats. Endocrinology., 99:(3), 652-658.

Drouva, S.V., Epelbaum, J., Tapia-Arancibia, L., La Plante, E., Kordon, C. (1980). Met-enkephalin inhibition of Potassium-induced LHRH and SRIF release from rat mediobasal hypothalamic slices. Eur.

J. Pharmacol. 61:411-413.

Drouva, S.V., Epelbaum, J., Tapia-Arancibia, L., La Plante, E., Kordon, C. (1981). Opiate receptors modulate LHRH and SRIF release from mediobasal hypothalamic neurons. Neuroendocrinol., 32:163-166.

Drouva, S.V., Gallo, R.V. (1976). Catecholaminergic involvement in episodic luteinizing hormone release in ovariectomized rats. Endocrinology., 99: 651-658.

Duggan, R.T., Bryant, M.J., Cunningham, F.J. (1982). Gonadotrophin, total oestrogen and progesterone concentration in the plasma of lactating sows with particular reference to lactational oestrus. J. Reprod. Fertil., 64:303-313.

Dusza, L., Krzymowska, H. (1979). Plasma prolactin concentration during the oestrous cycle of sow. J. Reprod. Fertil., 57:511-514.

Dusza, L., Tilton, J.E. (1990). Role of prolactin in the regulation of ovarian function in pigs. J. Reprod. Fertil., Suppl., 40:33-45.

Dusza., L. & Krzymowska, H. (1981). Plasma prolactin levels in sows during pregnancy, parturition and early lactation. J. Reprod. Fertil., 61:131-134.

Dyer, R.G. (1985). Neural signals for oxytocin and LH release. In: Oxford Review of Reproductive Biology. Ed. by J.R. Clarke. Oxford University Press. 7:223-260.

Dyer, R.G. (1988). Oxytocin and parturition new complications. J. Endocrinol., 116:167-168.

Ebling, F.J.P., Lincoln, G.A. (1985). Endogenous opioids and the control of seasonal LH secretion in Soay rams. J. Endocrinol., 107:341-353.

Ebling, F.J.P., Lincoln, G.A., Martin, G.B., Taylor, P.L. (1987). LHRH and beta-endorphin in the hypothalamus of the ram in relation to photoperiod and reproductive activity. Dom. Anim. Endocrinol., 4:149-156.

Ebling, F.J.P., Schwartz, M.L., Foster, D.L. (1989). Endogenous opioid regulation of pulsatile luteinizing hormone secretion during sexual maturation in the female sheep. Endocrinology., 125:369-383.

Edquist, L.E. Einarsson S. & Settergren, I. (1974). Ovarian activity and peripheral plasma levels of oestrogens and progesterone in the lactating sow. Theriogenology, 1:43-49.

Edwards, S. (1982). The endocrinology of the post partum sow. In: Control of Pig Reproduction. Ed. by Cole D.J.A. and Foxcroft, G.R.; Butterworths, London. pp. 439-458.

Edwards, S., Foxcroft, G.R. (1983). Endocrine changes in sows weaned at two stages of lactation. J. Reprod. Fertil., 67:161-172.

Elde, R., Hokfelt, T. (1979). Localization of hypophysiotropic peptides and other biologically active peptides within the brain. Anim. Rev. Physiol., 41:587-602.

Elsaesser, F., Parvizi, N. (1980). Partial recovery of the stimulatory oestrogen feed back action on LH release during late lactation in the pig. J. Reprod. Fertil., 59:63-67.

Esbenshade, K.L., Britt, J.H. (1990). Regulation and action of gonadotrophins in the pig. J. Reprod. Fertil., Suppl. 40:19-32.

Evans, G. (1980). Reproductive potential and endocrinological responses of sheep kept under controlled lighting.III.Prolactin and pituitary responsiveness to Gonadotrophin releasing hormone in the post partum period. Anim. Reprod. Sci., 3:57-68.

Farah., J.M., Sapum-Malcom, D., Muller, G.P. (1983). Dopaminergic inhibition of pituitary ß-endorphin-like immunoreactivity secretion in the rat. Endocrinology., 110:657-659.

Fang, V.S., Refetoff, S. (1978). Heterogenous human prolactin from a giant pituitary tumor in a patient with panhypopituitarism. J. Clin. Endocrinol. Metab., 47:780-787.

Fawcett, D.W., Hutchinson, J.S.M., Sharp, P.J. (1978). Gonadotrophic hormones; The hypothalamo-pituitary control of ovary. In: The Ovary. 2nd Edn., III. Ed. by S. Zuckerman, B.J. Weir. Academic Press N.Y., pp. 227-303.

Ferin, M., Van Vugt, D., Wardlaw, S. (1984). The hypothalamic control of the menstrual cycle and the role of endogenous opioid peptides. Rec. Prog. Horm. Res., 40:441-480.

Fernandes, L.C., Thatcher, W.W., Wilcox, C.J., Call E.P. (1978). LH release in response to GnRH during the post partum period of dairy cows. J. Anim. Sci., 46:443.

Findell, P.R., Wong, K.H., Jackman, J.K., Daniels, D.V. (1993). Beta1-adrenergic and dopamine (D1)-receptors coupled to adenylyl cyclase activation in GT1 gonadotropin-releasing-hormone neurosecretory cells. Endocrinology., 132:682-688.

Fink, G., (1988a). Oestrogen and progesterone interactions in control of gonadotrophin and prolactin release. J. Steroid. Biochem., 30:169-178.

Fink, G. (1988b). Gonadotrophin secretion and its control. In: The Physiology of Reproduction. Ed. by E. Knobil, J.D. Neill. 2:1349-1377.

Finnie, A.D., (1989). Opioid and dopamine modulation of luteinizing hormone secretion in live lambs. Ph.D. thesis, University of Nottingham.

Fitzgerald, B.P., Cunningham, F.J. (1981). Effect of removal of lambs or treatment with bromocriptine on plasma concentrations of prolactin and FSH during the post-partum period in ewe lambing at different times during the breeding season. J. Reprod. Fertil., 61:127-136.

Fletcher, I.C., (1973). Effect of lactation, suckling and oxytocin on post-partum oestrus in ewes. J. Reprod. Fertil., 333: 293- 298

Folkers, K., Fuchs, S., Humphries, J., Wan, Y.P., Bowers, C.Y. (1978). Follicle stimulating hormone releasing hormone.In: Novel aspects of reproductive physiology. Ed. by C.H. Spilman and J.W. Wilks. S.P. Medical and scientific books, New York, pp. 319-338.

Ford, J.J, Melampy, R.M. (1973). Gonadotrophin levels in lactating rats: effect of ovariectomy. Endocrinology., 93:540-547.

Forrest, D.W., Fleeger, J.L., Long, C.R., Soresen, A.M., Harms, P.G. (1980). Effects of exogenous prolactin on peripheral luteinizing hormone levels in ovariectomized cows. Biol. Reprod., 22: 197-201.

Foster, D.L. (1979). Is the mechanism for resumption of ovulation following parturition in the sheep similar to that for onset of puberty or onset of the adult breeding season? Biol. Reprod., 20:(suppl 1), 25 A.

Foster, D.L., Ebling, F.J.P., Vannerson, L.A., Wood, R.I. and Fenner, D.E.,1988. Regulation of puberty in the lamb: internal and external cues. In "Progress in Endocrinology" Eds. H. Imura, K. Shizume and S. Yoshida. Elsevier, Amsterdam. Vol.2:861.

Foster, D.L., Lemans, J.A., Jaffe, R.B., Niswender, G.D. (1975). Sequential patterns of circulating LH and FSH in female sheep during the early post-natal period: effect of gonadectomy. Endocrinology., 96:985-993.

Foxcroft, G.R, Pomerantz, D.K., Nalbandov, A.V. (1975). Effects of estradiol-17β on LHRH/FSH-RH induced and spontaneous LH release in prepubertal female pigs. Endocrinology., 96:(3) 551-557.

Foxcroft, G.R., Van de Wiel D.F.M. (1982). Endocrine control of the oestrous cycle. In: Control of Pig Reproduction. D.J.A. Cole and G.R. Foxcroft (ed.), Butterworth, London, pp.161-177.

Foxcroft G.R., Elsaesser, F. (1977). The ontogeny and characteristics of positive oestrogen feedback on luteinizing hormone secretion in the domestic female pig. J. Endocrinol., 75,(3):45

Foxcroft, G.R. (1985). Follicular dynamics and its endocrine control in the sow. In: Follicular growth and ovulation rate in farm animals. Eds. J.F. Roche and D. O'Callaghan. Martinus Nijhoff Publishers, pp.191-205.

Foxcroft, G.R., Shaw, H.J., Hunter, M.G., Booth, P.J., Lancaster, R.T. (1987). Relationship between luteinizing hormone, follicle stimulating hormone and prolactin secretion and ovarian follicular development in the weaned sow,. Biol Reprod. 36:175-191.

Foxcroft, G.R., Haresign, N.B., Haynes, N.B., Lamming, G.E., Peters, A.R. (1988). Gonadotropins-Domestic Animals. Acta Endocrinol., 288:41-50.

Franceschini, R., Venturini, P.L., Cataldi, A., Barreca, T., Ragni, N., Rolandi, E. (1989) Plasma beta-endorphin concentrations during suckling in lactating women. Br. J. Obst. Gynaec., 96:711-713.

Frowley, L.S., Miller, III, H.A. (1989). Ontogeny of prolactin secretion of the neonatal rat is regulated post-transcriptionally. Endocrinology., 124:3-6.

Fuke, K. & Hokfelt, T. (1966). Further evidence for the existence of tuberoinfundibular dopamine neurons. Acta Physiol. Scand., 66:245-246.

Garcia-Winder, M., Imakawa, K., Day, M.L., Zalesky, D.D., Kittok, R.J., Kinder, J.E. (1984). Effect of suckling and ovariectomy on the control of LH secretion during the post partum period in beef cows. Biol. Reprod., 31:771-778.

Garnier, P.F., Aubert, M.L., Kaplan, S.L. (1978). Heterogeneity of pituitary and plasma prolactin in man: Decreased affinity of pig prolactin in a radioreceptor assay and evidence for its secretion. J. Clin. Endocrinol. Metab., 47:1273-1281.

Gay, V.L., Plant, T.M. (1987). N-metyl-D, L-aspertate, elicits hypothalamic gonadotropin releasing hormone release in prepubertal male rhesus monkeys (Macaca Mulatta). Endocrinology., 120:2289-2296.

Genazzani, A.R., Petraglia, F. Nappi, G., Martignoni, E., De Leo, M., Facchinetti, F. (1984). Endorphins in peripheral plasma:origin and influencing factors. in: Central and peripheral endorphins: Basic and clinical aspects. Ed. by E.E. Muller and A.R. Genazzani, Raven press, New York. pp. 89-96.

Gerner, R.H., Sharp, B., Catlin, D.H. (1982). Peripherally administered beta-endorphin increases cerebrospinal fluid endorphin reactivity. J. Clin. Endocrinol. Metab., 55:358-360.

Giraud, P., Lissitzky, C., Devolix, B.C., Gilloz, P., Oliver, C. (1980). Influence of halperidol on ACTH and ß-endorphin secretion in the rat. Eur. J. Pharmacol., 62:215-217.

Glasier, A.F., McNeilly A.S., Howie, P.W. (1984). Pulsatile secretion of LH in relation to the resumption of ovarian activity post-partum. Endocrinology., 20:415-426.

Goding, J.R., Catt, K.J., Brown, J.M., Kailtenbach, C.C., Cumming, I.A., Mole, R.J. (1969). Radioimmunoassay for ovine luteinizing hormone. Secretion of luteinizing hormone during estrus and following estrogen administration in the sheep. Endocrinology., 85:133- 142.

Goldsmith, P.C., Cronin, M.J., Weiner, R.I. (1979). Dopamine receptor sites in the anterior pituitary. J. Histochem. Cytochem., 27:1205-1207.

Goodman, R.L., Karsh, F.J. (1980a). Pulsatile secretion of LH: differential suppression by ovarian steroids. Endocrinology., 107:1286-1290.

Goodman, R.R., Snyder, S.H., Kuhar, M.J., Young, W.S. (1980b). Differentiation of delta and mu opiate receptor localization by light microscopic autoradiography. Proc. Nat. Acad. Sci. USA, 77:6239.

Goodman, R.L., Bittman, E.L., Foster, D.L., Karsh, F.J. (1982). Alterations in the control of LH-pulse frequency underlie the seasonal variations in estradiol negative feed-back in the ewe. Biol. Reprod., 27:580-589.

Gordon, K., Renfree, M.B., Short, R.V., Clarke, L.I.J. (1987). Hypothalamo-pituitary portal blood concentrations of Beta-endorphin during suckling in the ewe. J. Reprod. Fertil., 79:397-408.

Grandison, C., Guidotti, A.A. (1977). Regulation of prolactin release by endogenous opiates. Nature, Lond., 270:357-359.

Gregg, D.W., Moss, G.E., Hudgens, R.E., Malven, P.V. (1986). Endogenous opioid modulation of luteinizing hormone and prolactin in post-partum ewes and cows. J. Anim. Sci., 63:838-847.

Grossman, A., West, S.W., Evans, J., Rees, L.H., Besser, G.M. (1982). The role of opiate in the control of prolactin in the puerperium, and TSH in primary hypothyroidism. Clin. Endocrinol., 16:317.

Grossman, A., Besser, G.M. (1984). In: Adolescence in female. Eds. Flamigni C., Venturoli S., Givens J.R. Year Book Medical Publisher, pp. 23-36.

Grossman, A. (1989). Opioid peptides, prolactin, and gonadotrophin regulation in the human. In: Brain opioid system in reproduction. Eds Dyer, R.G., Bicknell, R.J. Oxford University Press, 325-339.

Grossman, A., Dyer, R.G. (1989) Opioid inhibition of brainstem projections in the medial preoptic area in female rats. In: Brain opioid system in reproduction. Eds Dyer, R.G., Bicknell, R.J. Oxford University Press, 112-130.

Grosvenor, C.E., Whitworth, N.S. (1979). Secretion rate and metabolic clearance rate of prolactin in the rat during mid and late lactation. J. Endocrinol., 82:409- 415.

Hafez, E.S.E. (1984). Reproduction in farm animals. Lea and Febiger. Philadelphia, Pennsylvania.

Hagen, T.C., Arnaout, M.A., Scherzer, W.J., Martisn, D.R. and Garthwaute, T.L. (1986). Antisera to vasoactive intestinal peptide inhibit basal prolactin release from dispersed anterior pituitary cells. Neuroendocrinol., 43:641-645.

Hagen, T.C., Nangy, G., Mulchaney, J.J., and Neill, J.D. (1988). Autocrine control of hormone secretion in anterior pituitary cells. Endocrinology., 122:364-366.

Hall, T.R. (1984). Control of prolactin secretion in the vertebrates - A comparative survey. Gen. Pharmac., 15:189-195.

Hammer, R.P. Jr. (1985). The sex hormone-dependent development of opiate receptors in the rat medial preoptic area. Brain Res., 360:65-74.

Haynes, N.B., Lamming, G.E., Yang, K.P., Brooks, A.N., Finnie, A.D. (1989). Endogenous opioid peptides and farm animal reproduction. In: Oxford Reviews of Reproductive Biology. Ed. Millingan , S.R., 11:111-145.

Hoffman, B., Schams, D., Bopp, R., Ender, M.L., Gimenez, T., Karg, H.R. (1974). Luteotrophic factors in the cow: evidence for LH rather then PRL. J. Reprod. Fertil., 40:77-85.

Hoffman, G.E., Fitzsimmons, M.D., Watson, R.E. (1989). Relationship of endogenous opioid peptide axons and GnRH neurones in the rat. In Brain opioid systems in reproduction. Eds., R.G. Dyer, R.J. Bicknell. Oxford University Press. pp 124-134.

Hokfelt, T. and Fuke, K. (1972). Effect of prolactin and ergot alkaloids on the tubero-infundibular dopamine (DA) neurons. Neuroendocrinol., 9:100-122.

Horrobin, D.F. (1977). Prolactin, 5, Ed. by Churchill Livingstone, Edinburgh.
Horton, R.J.E., Jiang-Yuan Li, Cummins, J.T., A.I., Shen, P.J., Clarke, I.J. (1990). Morphine decreases LH secretion in ovariectomized Ewes only after steroid priming and not by direct pituitary action. Neuroendocrinol., 52:612-617.

Hutchinson, J.S.M., Sharp, P.J. (1978). Gonadotrophic hormones; The hypothalamo-pituitary control of ovary. In: The Ovary. 2nd Edn., III, Ed. Zuckerman, S., Weir, B.J. Academic Press N.Y., pp. 227-303.

Hyde, J.F., Murai, I., Ben-Jonathan, N. (1987). The posterior pituitary contains a potent prolacting-releasing factor: studies with perfused anterior pituitary cells. Endocrinology., 121:1531.

I'Anson, H., Ebling, F.J.P, Wood, R.I., Bucholtz, D.C., Foster, D.L. (1989). Potential for an opioidergic inhibition of GnRH release in the growth restricted hypogonadotropic lab. J. Reprod. Fert., Abstract series 4. Abstr. No. 6.

Ieiri, T., Chen, H.T., Meites, J. (1980). Naloxone stimulation of luteinizing hormone release in prepubertal females rats, role of serotonergic system. Life Sci., 26:1269-1274.

Ishibashi, T., Shiino, M. (1989). Co-localization pattern of GH and PRL within the anterior pituitary cells in the female rat and female musk shrew. Anat. Rec. 233(2):185-193.

Jackson, G.L., Kuehl, D., McDowell, K., Zaleski, A. (1978). Effect of hypothalamic deafferentation on secretion of LH in the ewe. Biol. Reprod., 18:808.

Jacobs, L., Snyder, P.J., Wilber, J.F., Utiger, R.D. Daughaday, W.H. (1971). Increased serum prolactin after administration of synthetic thyrotropin releasing hormone. J. Clin. Endocrinol. Metab., 33:996-998.

Jakuboswski, M., Terkel, J. (1985). Role of nipple stimulation in the suppression of estrus cyclicity during extended lactation in the rat. Biol. Reprod., 33:920-924.

Jagger, J.P., Peters, A.R., Lamming, G. E. (1987). Hormone responses to low dose GnRH treatment in post partum beef cows. J. Reprod. Fertil., 80:263-269.

Jackson, J.L. (1977). Effect of adrenergic blocking drugs on secretion of LH in the ovariectomized ewe. Biol. Reprod., 16:543-548.

James, M.D., MacKenzie, F.J., Tuohy-Jones, P., Wilson, C.A. (1987). Dopaminergic neurones in the zona incerta exert a stimulatory control on gonadotrophin release via D1 dopamine receptors. Neuroendocrinol., 45:348.

Jenkin, G., Heap, R.B. (1974). The lack response of the sheep pituitary to LHRH stimulation in gestation and early lactation; the probable role of progesterone. J. Endocrinol., 61, XII-XIII.

Jenkin, G., Heap, R.B., Symons, D.B.A. (1977). Pituitary responsiveness to synthetic LHRH and pituitary LH content at various reproductive stages in the sheep. J. Reprod. Fertil., 49:207-214.

Johns, M.A., Azmita, E.C., Kreiger, D.T. (1982). Specific in vitro uptake of serotonin by cells in the anterior pituitary of the rat. Endocrinology., 110:256-263.

Joseph, I.B.J., Currie, W.D., Rawlings, N.C. (1992). Effects of time after ovariectomy, season and oestradiol on luteinizing hormone and follicle-stimulating hormone secretion in ovariectomized ewes. J. Reprod. Fertil., 4:511-523.

Kaji, H., Chihara, K., Abe, H., Kita, T., Kashio, Y., Okimura, Y., Fuita, T. (1985). Effect of passive immunization with antisera to vasoactive intestinal polypeptide and peptide histidine isoleucine amide on 5-Hydroxy-l-tryptophan-induced prolactin release in rats. Endocrinology., 117, 1914-1919.

Karla, S.P. (1981) Neural loci involved in naloxone-induced luteinizing hormone release: Effects of norepinephrine synthesis inhibitor. Endocrinology., 109:1805-1810.

Kalra, S.P., Kalra, P.S. (1984). Opioid-adrenergic-steroid connection in regulation of luteinizing hormone secretion in the rat. Neuroendocrinol., 38:418-426.

Kalra, S.P., Crowley, W.R. (1984) Norepinehprine-like effects of neuropeptide Y on LH release in the rat. Life Sci., 35:1173-1176.

Kalra, S.P., Simpkins, J.W. (1981). Evidence for noradrenergic mediator of opioid effect on LH secretion. Endocrinology., 109:776-782.

Kalra, S.P., (1983). Opioid peptides-inhibitory neuronal system in regulation of gonadotrophin secretion. In: Role of peptides and proteins in control of reproduction. Eds., McCann, S.M., Dhindsa, D.S., Elsevier, pp. 63-87.

Kamberi, I.A., Mical, R.S., Porter, J.C. (1971). Effect of melatonin and serotonin on release of FSH and prolactin. Endocrinology., 88:1288-1293.

Kanchev, L.N., Dobson, H., Stankov, B.M. (1984). Early pituitary luteinizing hormone response to injections of gonadotrophin releasing hormone in ewes. Theriogenology, 21:407-417.

Kann, G., Martinet, J. (1975). Prolactin levels and duration of post-partum in anoestrus lactating ewes. Nature,257:63-64.

Kann, G., Martinet, J., Schirar, A. (1977). Hypothalamic- pituitary control during lactation in the sheep. In: Control of ovulation. Eds., D.B. Crighton, N.B., Haynes, G.R. Foxcroft and G.E. Lamming. Butterworths, London pp. 19-34.

Karsch, F.J., Legan, S.J., Hauger, R.L., Foster, D.L. (1977). Negative feedback action of progesterone on tonic luteinizing hormone secretion in the ewe: dependence on the ovaries. Endocrinology., 101:800-806.

Karsch, F.J., Legan, S.J., Hauger, R.L., Foster, D.L. (1977). The feedback effect of ovarian steroid on gonadotrophin secretion. In: Control of ovulation. Eds., D.B. Crighton, G.R. Foxcroft, N.B. Haynes and G.E. Lamming. Butterworths, London. pp 29-48.

Karsch, F.J., Foster, D.L. (1975). Sexual differentiation of the mechanism controlling the preovulatory discharge of LH in the sheep. Endocrinology., 97:373-379.

Karsch, F.J., Goodman, R.L., Legan, S.J. (1980) Feed-back basis of seasonal breeding: test of an hypothesis. J. Reprod. Fertil., 58:521-535.

Kebabian, K.W., Calne, D.B., (1979). Multiple receptors for dopamine. Nature, Lond. 277:93-96.

Kessler, D.J., Garverick, H.A, Youngquist, R.S., Elmore, R.G. Bierschwal, C.J. (1977). Effect of days post partum and endogenous reproductive hormones on GnRH-induced LH release in dairy cows. J. Anim. Sci., 45:797-803.

Kesner, J.S., Kaufman, J.M., Wilson, R.C., Kuroda, G., Knobil, E. (1986). The effect of morphine on the electrophysiological activity of the hypothalamic luteinizing hormone releasing hormone pulse generator in the resus monkey. Neuroendocrinol., 43:686-688.

Kesner, J.S., Estienne, M.J., Kraeling, R.R., Rampacek, G.B. (1989). LH and Prolactin secretion given estradiol and pulsatile GnRH in hypophysial stalk-transected pigs. Neuroendocrinol., 49:502-508

Keye, W.R. Jr., Jaffe, R.B. (1976). Changing patterns of FSH and LH response to gonadotrophin releasing hormone in the puerperium. J. Clin. Endocrinol. Metab. 42:1133-1138.

King, J.C., Tobet, S.A., Snavely, F.L., Arimura, A.A. (1982). LHRH immunopositive cells and their projections to the median eminence and organum vasculosum of the lamina terminalis. J. Comp. Neur., 209:287-300.

Khorram, O., Pau, F.K.I., Spies, H.G.(1987). Bimodal effect of neuropeptide Y on hypothalamic release of GnRH in conscious rabbits. Neuroendocrinol., 45:290-297.

Knight, P.G., Howles, C.M., Cunningham, F.J. (1986). Evidence that opioid peptides and dopamine participate in the suckling induced release of prolactin in the ewe. Neuroendocrinol., 14:29-35.

Koening, J.I., Mayfield, M.A., McCann, S.M., Krulich, L. (1980a). Stimulation of prolactin secretion by morphine: role of the central serotonergic system. Life Sci. 25:853-855.

Koening, J.I., Mayfield, M.A., Coppings, R.J., McCann, S.M., Krulich, L. (1980b). Role of central nervous system neurotransmitters in mediating the effects of morphine on growth hormone and prolactin secretion in the rat. Brain Res., 10:453-455.

Koening, J.I., Krulich L. (1982). Differential role of multiple opioid receptors in the regulation of secretion of prolactin and growth hormone in rats. In: Opioid modulation of endocrine function. Ed. G. Delitalia, M. Motta and M.Serio. Raven, New York pp.89-98.

Koening, I.J., Mayfield, M.A., McCAnn, S.M., Kruhlich, L. (1984). Differential role of the opioid mu and kappa receptors in the activation of prolactin and growth hormone secretion of morphine in male rat. Life Sci., 34:1829-1837.

Kordon, C., Javoy, F., Vassent, G., Glowinski, J. (1968). Blockade of superovulation in the immature rat by increased brain serotonin. European J. Pharmac., 4:169-174.

Kordon, C., Blake, C.A., Terkel, J., Sawyer, C.H. (1973). Participation of serotonin-containing neurons in the suckling-induced rise in plasma prolactin levels in lactating rats. Neuroendocrinol., 13:213-223.

Kraeling, R.R., Rampacek, G.B. (1986). Serum prolactin response to thyrotropin releasing hormone in estradiol treated hypophysial stalk-sectioned gilts. Domest. Anim. Endocrinol., 3:289-94

Kraeling, R.R., Kesner, J.S., Estienne, M.J., Barb, C.R., Rampacek, G.B. (1987). Follicular growth after pregnant mare serum gonadotropin (PMSG) in hypophysial stalk-transected (HST) pig given pulsatile GnRH. J. Anim. Sci., 3:177-183.

Kraeling, R.R., Barb, R.C., Rampacek, GB. (1992). Prolactin and luteinizing hormone secretion in the pregnant pig. J. Anim. Sci. 70:3521-3527.

Kraeling, R.R., Rampacek, G.B., Cox, N.M., Kiser, T.E. (1982). Prolactin and luteinizing hormone secretion after bromocryptine (CB 154) treatment in lactating sows and ovariectomized gilts. J. Anim. Sci., 54:1212-1220.

Krulich, L. (1979). Central neurotransmitters and the secretion of PRL, GH, LH and TSH. Ann. Rev. Physiol., 41:603-615.

Kuljis, R.O., Advis, J.P. (1989). Immunocytochemical and physiological evidence of a synapse between dopamine and luteinizing hormone releasing hormone-containing neurons in the ewe median eminence. Endocrinology., 124:1579-1581.

Kunavongkrit, A., Kindahl, H., Einarsson, S., Edquist, L.E. (1983). Clinical and endocrinological studies in primiparous zero-weaned sows. 1) Clinical and morphological findings with special reference to the effect of PGF-2α treatment. Zbl. Vet. Med., 30:607-615.

Labrie, F., Lagace, L., Drouin, L., Ferland, L., Kelly, P.A., Cusan, L. (1978). Specific modulation of LHRH action on LH and FSH secretion by gonadal steroids. In: Control of Ovulation. Eds., Crighton DB, Haynes NB, Foxcroft GR, Lamming GE, Butterworths, London, 73-86.

Laedem, C.A., Kalra, S.P. (1985). Effects of endogenous opioid peptides and opiates on LH and prolactin secretion. Neuroendocrinol., 41:342-352.

Laedem, C.A., Yagenova, S.V. (1987). Effects of specific activation of mu- delta- and kappa-opioid receptors on the secretion of luteinizing hormone and prolactin in ovariectomized rat. Neuroendocrinol., 45:109-117.

Lantz, W.B., Zimmerman, D.R. (1972). Plasma luteinizing hormone levels in estradiol treated ovariectomized gilts. J. Anim. Sci., 35:1120 Abstr.

Lauderdale, J.M., Kirkpatrick, R.L., First, N.L., Hauser, E.R., Casida, L.E. (1965). Ovarian and pituitary gland changes in periparturient sows. J. Anim. Sci., 24:1100-1103.

Lawson, D.M., Gala, R.R. (1975). The influence of adrenergic, dopaminergic, cholinergic and serotonergic drugs on plasma prolactin levels in ovariectomized, estrogen-treated rats. Endocrinology., 96:313-318.

Leakakos, T., Hudgens, R.E., Diekman, M.A., Moss, G.E. (1987). Effects of estradiol-17beta and gonadotropin releasing hormone on post-partum secretion of luteinizing hormone in fall lambing ewes. J. Anim. Sci., 64:1484-1490.

Leebaw, W.F., Lee, L.A., Woolf, P.D., (1978). Dopamine effect on basal and augmented pituitary hormone secretion. J. Clin. Endocrinol. Metab., 47:480.

Lehman, M.N., Robinson, J.E., Karsh, F.J., Silverman, A.J. (1986). Immunocytochemical localization of LHRH pathways of the sheep brain during anestrus and the midluteal phase of the estrus cycle. J. Comp. Neurol., 244:1935.

Leibowitz, S.F., Jhanwar-Uniyal, M., Dvorkin, B., Makman, M.H. (1982). Distribution of alfa adrenergic, beta-adrenergic and dopaminergic receptors in discrete hypothalamic areas of the rat. Brain. Res., 233:97.

Levine, J.E., Pav, K.Y., Ramirez, V.D., Jackson, G.L. (1982). Simultaneous measurements of luteinizing hormone-releasing hormone and luteinizing hormone release in unanaesthetized, ovariectomized sheep. Endocrinology., 111:1444-1449.

Levine, J.E., Norman, R.L., Ghessman, P.M., Oyama, T.T., Bangsber, D.R., Spies, H.G. (1985). In vivo gonadotropin-releasing hormone release and serum luteinizing hormone measurements in ovariectomized, estrogen-treated rhesus monkeys. Endocrinology., 117:711-721.

Lewis, U.J. (1975). Chemistry of prolactin. Pharmacol. Ther., 1:423-435.

Lincoln, G.A., Ebling, F.J.P. and Martin, G.B., (1987). Endogenous opioid control of pulsatile LH secretion in rams: modulation by photoperiod and gonadal steroids. J. Endocrinol., 115:425-438.

Ling, N., Ling, S.Y., Ueno, N., Esch, F., Denoroy, L., Guillemin, R., (1985). Isolation and partial characterization of a Mr 32.000 protein with inhibin activity from porcine follicular fluid. Proc. Natl. Acad. Sci., USA. 82:7217-7221.

Lishman, A.W., Allison, S.M.J., Fojwell, R.L., Butcher, R.L., Inskeep, E.K. (1979). Follicular development and function of induced corpora lutea in underfed post partum anestrous beef cows. J. Anim. Sci., 48:867-875.

Lord, J.A.H., Waterfeld, A.A., Hughes, J., Kosterlitz, H.W. (1977) Endogenous opioid peptide: multiple agonist and receptors. Nature, 267:495-499.

Lu, K.F., Chen, H.T., Huang, H.H., Grandison, L., Marshall, S., Meites, J. (1976). Relation between prolactin and gonadotropin secretion in post-partum lactating rats. J. Endocrinol., 68:241-250.

Lumpkin, M.D., McCann, S.M. (1984). Effect of destruction of the dorsal anterior hypothalamus on follicle-stimulating hormone secretion in the rat. Endocrinology., 115:2473-2480.

Lumpkin, M.D., Samson, W.K., McCann, S.M. (1983). Hypothalamic and pituitary sites of action of oxytocin to alter prolactin secretion in the rat. Endocrinology., 112, 1711.

Lumpkin, M.D., McDonald, J.K., Samson, W.K., McCann, S. (1989). Destruction of the dorsal anterior hyphothalamic region suppresses pulsatile release of follicle stimulating hormone but not luteinizing hormone. Neuroendocrinol., 50:229-235.

Lux-Lantos, V., Rey, E., Libertun, C. (1992) Activation of GABA B receptors in the anterior pituitary inhibits prolactin and LH secretion. Neuroendocrinol., 56:687-93.

MacDonald, R.D., Peters, J.L. and Deaver, D.R.,1990. Effect of naloxone on the secretion of LH in infantile and prepubertal Holstein bull calves. J. Reprod. Fertil., 89:51-57.

Macleod, R.M., Judd, A.M., Spangelo, B.L., Ross, P.C., Jarvis, W.D. and Login, I.S. (1988). Systems that regulate prolactin release. In: Prolactin gene family and its receptors. Ed. K. Hoshino. Elsevier Science Publisher, pp. 13-27.

Malven, P., (1986). Inhibition of pituitary LH release resulting from endogenous opioid peptides. Dom. Anim. Endocrinol., 3:135-144.

Martin, G.B. (1984). Factors affecting the secretion of LH in the ewe. Biol. Rev. 9:1-87.

Martin, W.H., Rogol, A.D., Kaiser, D.L., Thorne, M.O. (1981). Dopaminergic mechanisms and luteinizing hormone (LH) secretion. II Differential effects of dopamine and bromocriptine on LH release in normal women. J. Clin. Endocrinol. Metab., 52:650-656.

Martin, G.B., Scaramuzzi, R.J., Henstridge, J.D. (1984). Effects of estradiol, progesterone and androstenedione on the pulsatile secretion of LH in ovariectomized ewes during spring and autumn. J. Endocrinol., 96:181-193.

Martinez-de-la-Escalera, G., Gallo, F., Choi, A.L., Weiner, R.I., (1992) Dopaminergic regulation of the GT1 gonadotropin-releasing hormone (GnRH) neuronal cell lines; stimulation of GnRH release via D1-

receptors positively coupled to adenylate cyclase. Endocrinology., 131:2965-71.

Masotto, C., Negro-Vilar, A., (1987). Activation of gamma-amino butyric acid beta receptors abolishes naloxone stimulated luteinizing hormone release. Endocrinology., 121:2251-2255.

Masotto, C, Wisniewski, G, Negro-Villar, A. (1989) Different gamma-aminibutyric acid beta-receptors subtypes are involved in the regulation of opiate-dependent and independent luteinizing hormone-releasing hormone secretion. Endocrinology., 125: 548-553.

Mathji, J.A.M., Gruisen, E.R.M., Swaets, J.J.M. (1979). The suckling-induced rise of plasma prolactin in lactating rats: its dependence on stage of lactation and litter size. Horm. Res., 11, 325-336.

Matteri, R.L., Moberg, G.P., (1985). The effect of opioid peptides on ovine pituitary gonadotropin secretion in vitro. Peptides, 6:957-963.

Matthews, M.V., and Murdoch, W.J., (1984). Increased secretion of luteinizing hormone in prepubertal ewes treated with an antagonist of opiates. Dom. Anim. Endocrinol., 1:167-163.

Mattioli, M., Seren, E. (1985). Effect of bromocriptine treatment during lactational anestrous in the pig. In: Endocrine causes of seasonal and lactational anestrous in farm animals. Ed. Ellendorff, F., Elasaesser, F., Martinus-Nijhoff Pub., pp. 165-178.

Mattioli, M., Conte, F., Seren, E., Galeati, G. (1986). Effect of naloxone on plasma concentrations of prolactin and LH in lactating sows. J. Reprod, Fertil., 76:167-173.

McLeod, B.J., Haresign, W., Lamming, G.E. (1982). The induction of ovulation and luteal function in seasonally anoestrous ewes treated with small-dose multiple injection of GnRH. J. Reprod. Fertil., 65:215-221.

McNatty, K.P., Sawers, R.S. & McNeilly, A.S. (1974). A possible role for prolactin in control of steroid secretion by the human graafian follicle. Nature, London., 250:635-655.

McNeilly, A.S., Glasier, A., Jonassen, J., Howeic, P.W. (1982). Evidence for a direct inhibition of ovarian function by PRL. J. Reprod. Fertil., 65:559-569.

McNeilly, A.S., Baird, D.T (1983a). Direct effect of prolactin induced by TRH injection on ovarian oestradiol secretion in the ewe. J. Reprod. Fertil., 69:559-568.

McNeilly, A.S., Sharpe, R.M. and Fraser, H.M. (1983b). An increased sensitivity to negative feed-back effects of testosterone induced by hyperprolactinaemia in the adult male rat. Endocrinology., 112:22-28.

McNeilly, A.S. (1987). Prolactin and the control of gonadotrophin secretion. J. Endocrinol., 115:1-5.

McNeilly, A.S. (1988). Suckling and the control of gonadotrophin secretion. In: The Physiology of Reproduction. Eds., E. Knobil, J.D. Neill. 2:2323-2349.

Meites, J. (1988). Biological functions of prolactin in mammals. In: Prolactin gene family and its receptors. Ed. K. Hoshino. Elsevier Science Publisher. pp. 123-130.

Melampy, R.M., Henricks, D.M., Anderson, L.L., Chen, C., Schultz, I.R. (1966). Pituitary follicle-stimulating hormone and luteinizing hormone concentrations in pregnant and lactating pigs.

Endocrinology., 78:801-804.

Mena, F., Martinez-Escalera, G., Clapp, C., Aguayo, D., Crosvenor, C.E. (1984). Regulatory and control mechanisms of prolactin secretion within the pituitary of the lactating rat. In: Prolactin secretion: A multidisciplinary Approach. Ed. F. Mena and R. Valverde. Academic Press, New York. pp. 161-185

Mena, F., Enjalbert, L. Carbone, H., Priam M., Kordans (1976). Effect of suckling on plasma prolactin and hypothalamic monoamine levels in the rat. Endocrinology., 99:445-451.

Meyer, S.L., Goodman, R.L. (1985). Neurotransmitters involved in mediating the steroid dependent suppression of pulsatile LH secretion in anestrous ewes; effects of receptor antagonists. Endocrinology., 116:2054-2161.

Meyer, S.L., Goodman, L.R. (1986). Separate neural system mediate the steroid-dependent and steroid-independent suppression of tonic luteinizing hormone secretion in the anestrous ewe. Biol. Reprod., 35:562-571.

Mezey, E. and Palkovits, M. (1982). Two-way transport in the hypothalamo-hypophyseal system. In: Frontiers in Neuroendocrinology. Ed. W.F. Ganong and L. Martini. Raven Press, New York, pp. 1-14.

Miller, W.L., Knight, M.M., Crimek, M.J., Gorski, J. (1977). Estrogen regulation of follicle stimulating hormone in cell cultures of sheep pituitaries. Endocrinology., 100:1306-1316.

Milmore, J.B., Reece, R.P. (1975). Effect of porcine hypothalamic extract on prolactin release in the rat. Endocrinology., 96:732-738.

Miyachi, V., Meklenburg, R.S., Lipsett, M.B. (1973). In vitro studies of pituitary median eminence unit. Endocrinology., 93:492-496.

Miyake, A., Tahizawa, O., Kurachi, K. (1978). Pituitary luteinizing hormone response to LHRH during puerperium. Obstet. Gynecol., 51:37.

Montgomery, G.W. (1982). Influence of suckling frequency and bromocriptine treatment on the resumption of ovarian cycles in postpartum beef cattle. Theriogenology, 17:551-563.

Montgomery, G.W., Martin, G.B., Pelletier, J. (1985). Changes in pulsatile LH secretion after ovariectomy in the Ile-de-France ewes in two seasons. J. Reprod. Fertil. 73:173-183.

Moore, K.E., Demarest, K.T. (1982). Tuberoinfundibular and tuberohypophyseal dopaminergic neurones. In: Frontiers in Neuroendocrinol., 161-190. Raven Press, New York

Morley, J.E. (1980). Endocrine effects of naloxone-induced opiate receptor blockade. J. Clin. Endocrinol. Metab. 50:251-257.

Morrel, J.I., McGinty, J.F., Pfaff, D.W. (1985). A subset of β-endorphin or dynorphin containing neurons in the medial basal hypothalamus accumulates estradiol. Neuroendocrinol., 41:417-426.

Moss, G.E., Adams, T.E., Niswender, G.D., Nett, T.M. (1980). Effect of parturition and suckling on concentration of pituitary gonadotropins, hypothalamic GnRH and pituitary responsiveness to GnRH in ewes. J. Anim. Sci., 50:496-502.

Moss, G.E., Crowder, M.E., Nett, T.M. (1981). GnRH receptor interaction: Effect of progesterone and estradiol on hypophyseal concentration of gonadotropins in ovariectomized ewes. Biol. Reprod., 25:938-944.

Moss, G.E, Parfet, J.R., Marvin, G.R., Allrich, R.D., Diekman, M.A. (1985). Pituitary concentrations of gonadotropins and receptors for GnRH in suckled beef cows at various intervals after calving. J. Anim. Sci., 60:285-293.

Moyer, J.A., O'Donohue, T.A., Herrenkohl, L.R., Gala, R.R., Jacobowitz, D.M. (1979) Effects of suckling on serum prolactin levels and catecholamine concentrations and turnover in discrete brain regions. Brain Res., 176:124-133.

Murai, I., Ben Johnathan, N. (1987). Posterior pituitary lobectomy abolishes the suckling-induced rise in prolactin: evidence for a prolactin-releasing factor in posterior pituitary. Endocrinology., 121:205-211.

Nagy, G., Mulchahey, J.J., Neill, J.D. (1988). Autocrine control of prolactin secretion by vasoactive intestinal peptide. Endocrinology., 122, 364-366.

Nett, T.M., Akbar, A.M., Niswender, G.D., Hedlund, M.T., White, W.F. (1973). A radioimmunoassay for gonadotrophin-releasing hormone (GnRH) in serum. J. Clin. Endocrinol. Metab., 36:880-885.

Nett, T.M. (1987). Function of the hypothalamic-hypophysial axis during the post-partum period in ewes and cows. J. Reprod. Fertil., 34:201-213.

Newton, G.R., Schillo, K.K. and Edgerton, L.A., (1988). Effects of naloxone on luteinizing hormone secretion in postpartum ewes. Biol. Reprod.,39:532-535.

Niall, H.D. (1981). The chemistry of prolactin. In: Prolactin. Ed. B. Elsevier Publisher, pp. 1-13.

Nillius, S.J., Wide, L. (1971). Induction of a midcycle-like peak of luteinizing hormone in young woman by exogenous oestradiol-17beta. J. Obstet. Gynaecol. Br. Commonwealth, 78:822-827.

Nikolics, K., Mason, A.J., Sronyii, I., Ramachandran, K., Soeburg, P.H. (1985). A prolactin-inhibiting factor within the precursor for human gonadotropin-releasing hormone. Nature, 316:511-517.

Nunez, E.A., Gerson, M.D., Silverman, A. (1981). Uptake of 5-hydroxytryptamine by gonadotrophs of the bat's pituitary: a combined immunocytochemical radioautographic analysis. J. Histochem. Cytochem., 29:1336-1346.

Padmanabdan, V., Kesner, J.S., Convey, E.M. (1978). Effects of estradiol on basal and luteinizing hormone releasing hormone (LHRH)-induced release of luteinizing hormone (LH) from bovine pituitary cells in culture. Biol. Reprod., 18:603-607

Page, R.B. (1983). Directional pituitary blood flow: a microcine-photographic study. Endocrinology., 112:157-165.

Palkovits, M., Arimura, A., Brownstein, M., Schally, A.V., Saaedra, J.M. (1974). Luteinizing hormone-releasing hormone (LH-RH) content of the hypothalamic nuclei in rats. Endocrinology., 95:554-558.

Panerai, A., Petraglia, F., Sacerdote, P., Genazzani, A.R. (1985). Mainly mu opiate receptors are involved in LH and prolactin secretion Endocrinology., 117:1096-1099.

Pang, C.N., Zimmerman, E., Sawyer, C.H. (1977). Morphine inhibition of the preovulatory surge of plasma luteinizing hormone and follicle stimulating hormone. Endocrinology., 101:1726-1732.

Parlow, A.F., Daane, T.A., Dignam, W.J. (1970) On the concentrations of radioimmunoassayable FSH circulating in blood during human pregnancy. J. Clin. Endocrinol. Metab., 31: 213-214.

Parvizi, N., Elsaesser, F., Smidt, D., Ellendorff, F. (1976). Plasma luteinizing hormone and progesterone in the adult female pig during the oestrous cycle, late pregnancy and lactation, and after ovariectomy and pentobarbitone treatment. J. Endocrinol., 69:193-203.

Paterson, S.J., Robson, L.E., Kosterliz, H.W. (1983). Classification of opioid receptors. British Medical Bulletin, 39:31-36.

Peters, A.R., Lamming, G.E., Fisher, M.W. (1981). A comparison of plasma LH concentrations in milked and suckling post-partum cows. J. Reprod. Fertil., 62:567-573.

Peters, A.R. (1984). Effect of exogenous oestradiol-17-β on gonadotrophin secretion in post partum beef cows. J. Reprod. Fertil., 72:473.

Petersen, S.L., Barraclough, C.A. (1986). Interaction between the hypothalamic opiate and catecholamine systems in the regulation of LH and prolactin secretion, In: Neuroendocrine perspectives, Eds. E.E. Muller, R.M. McLeod, 5:283-290.

Pfeiffer, D.G., Pfeiffer, A., Shimahigashi, K., Merriam, G.R., Loriaux, D.L. (1983). Predominant involvement of mu rather than delta or kappa opiate receptors in LH secretion. Peptides 4:647-649.

Plotsky, P.M., Neill, J.D. (1982). The decrease in hypothalamic dopamine secretion induced by suckling: comparison of voltammetric and radioisotopic methods of measurement. Endocrinology., 110:691-696.

Pontionroli, A.E., Pozza, G. (1978). Histamine stimulates prolactin release in normal men. Acta Endocrinol., 88:23-28.

Pontiroli, A.E., Viberti G.C., Mangili, R., Cammelli, L., Orlandini, G., Pozza, G., Dubuni, A. (1985). Long lasting prolacting-lowering effect of FCE 21336 in man. J. Endocrinol. Invest., Suppl.3 8:103.

Pope, G.S. (1982). Oestrogen and progesterone in plasma and milk of post partum dairy cattle. In: Factors influencing fertility in the post partum cow. Ed. by H. Karg E.Schallenberger. Martinus Nijhoff, Hague, pp. 248-276.

Prunier, A., Ellendorff, F. and Parvizi, N., (1990). Opioid action on luteinizing hormone secretion in newborn pigs: paradoxical effects of naloxone. J. Develop. Physiol., 14:221-227.

Przekop, F., Skubiszewsky, B., Wolinska, E., Domanski, E. (1975). The role of catecholamines in stimulating the release of pituitary ovulatory hormone in sheep. Acta Physiol., 85:481-486.

Quigley, M.E., Yen, S.S.C. (1980). The role of endogenous opiates on LH secretion during the menstrual cycle. J. Clin. Endocrinol. Metab., 51:179-181.

Ramirez, V.D., Sawyer, C.H. (1965). Advancement of puberty in the female rat by estrogen.

Endocrinology., 76:1158-1168.

Ramirez, V.D., Feder, H.H., Sawyer, C.H. (1984). The role of brain catecholamines in the regulation of LH secretion: a critical inquiry. Frontiers in Neuroendocrinol., 8:27-83.

Ramirez, J.L., Bennet, W.A., Cox, N.M. (1985a). Effect of stage of lactation on estrous behaviour, LH and ovulation after administration of estradiol benzoate to sows. J. Anim. Sci., 61:406 Abstr..

Ramirez, J.L., Bennet, W.A., Cox, N.M. (1985b). Characterization of oestrus and ovulation in lactating sows given pulsatile gonadotrophin-releasing hormone terminated at oestrus or 24h later. J. Anim. Sci., 61:44, abstr.

Rasmussen, D.D., Liu, J.H., Wolf, P.L., Yen, S.S.C. (1986). GnRH neurosecretion in the human hypothalamus. In vitro regulation by dopamine. J. Clin. Endocrinol. Metab., 62:479.

Rawlings, N.C., Jeffcoate, I.A., Rieger, D.L. (1984). The influence of oestradiol-17beta and progesterone on peripheral serum concentration of luteinizing hormone and follicle stimulating hormone in the ovariectomized ewe. Theriogenology 22:473-488.

Rawlings, N.C., Churchill, I.J. (1990) Effect of naloxone on gonadotrophin secretin at various stages of development in ewe lamb. J Reprod. Fertil., 89:503-509.

Rawlings, N.C., Currie, W.D., Evans, A.C.O, Churchill, I.J. (1993). Opioid modulation of gonadotropin secretion in mature ewe during the breeding season and seasonal anestrus and in the prepubertal ram, bull, ewe and heifer. In: Opioids in farm animals, Eds., N.Parvizi, Landwirtschaftsverlag GmbH, 4400 Münster-Hiltrup, Germany. pp. 77-98.

Rawlings, N.C., Churchill, I.J., Currie, W.D., Joseph, I.B. (1991). Maturational changes in opioidergic control of luteinizing hormone and follicle stimulating hormone in ram lambs. J. Reprod. Fertil., 93:1-7.

Rawlings, N.C., Weir, L., Todd, B., Manns, J., Hyland, J.H. (1980). Some endocrine changes associated with the post partum period of the suckling beef cow. J. Reprod. Fertil., 60:301-308.

Rayford, P.L., Brinkley, H.J., Young, E.P. (1974). Radioimmunoassay of porcine FSH. J. Anim. Sci., 39:348-354.

Reid, R.L., Hoff, J.D., Yen, S.C., Li, C.H. (1981). Effect of exogenous β endorphin on pituitary hormone secretion and its disappearance rate in normal human subjects. J. Clin. Endocrinol. Metab., 52:1179-1184.

Riley, G.M., Peters, A.R., Lamming, G.E. (1981). Induction of pulsatile LH release, FSH release and ovulation in post partum acyclic beef cows by repeated small doses of GnRH. J. Reprod. Fertil., 63:559-565.

Riskind, P.N., Millard, W.j., Martin, J.B. (1984) Opiate modulation of the anterior pituitary hormone response during suckling in the rat. Endocrinology., 114:1232-1237.

Rivier, C., Vale, N., Ling, N., Brown, M., Guillemin, R. (1977). Stimulation in vivo of the secretion of prolactin and growth hormone by β-endorphin. Endocrinology., 100:238-241.

Robertson, H.A., King, G.J. (1974). Plasma concentrations of progesterone, oestrone, oestradiol-17-β and oestrone sulphate in the pig at implantation, during pregnancy and at parturition. J. Reprod. Fertil., 40:133-141.

Robyn C., Meuris, S. (1988) The state of the art in human prolactin. In: Prolactin gene family and its receptors. Ed. by K. Hoshino. Elsevier Science Publisher, pp. 29-39.

Rodriguez-Sierra J.F., Balke C.A. (1982). Catecholestrogens and release of anterior pituitary gland hormones: Prolactin. Endocrinology., 110:325-329.

Rojanasthien, S.A.M., Lundeheim, N., Einarsson, S. (1987). Luteinizing hormone response to different doses of synthetic gonadotrophin releasing hormone during early and late lactation in primiparous sows. Anim. Reprod. Sci., 13:299-307.

Rolland R., Hammond, J.M. (1975a). Demonstration of a specific receptor for prolactin in porcine granulosa cells. Endocr. Res. Commun., 2:281-298.

Rolland, R., Lequin, R.M., Schellekens, L.A., De Jong F.H. (1975b). The role of prolactin in the restoration of ovarian function during the early post partum period in the human female I. A study during physiological lactation. Clin. Endocrinol., 4:15-25.

Rosen, J.M., Matusik, R.J. Richards, D.A., Gupta, P., Rodgers, J.R.,(1980) Multihormonal regulation of casein gene expression at the transcriptional and posttranscriptional levels in the mammary gland. Rec. Prog. Horm. Res. 36, 157-187.

Rossi, A., Disalle, E., Briatico, G., Arcari, G., De Castiglione, R., Perseo, G. (1983). Antinociceptive, prolactin releasing and intestinal motility inhibiting activities of dermorphin and analogues after subcutaneous administration in the rat. Peptides, 4:577-580.

Rotsztejn, W.H., Drouva, S.V., Pattou, E., Kordon, C. (1978). Met-enkephalin inhibits in vitro dopamine-induced LHRH release from mediobasal hypothalamus of male rats. Nature, 274:281-283.

Rotsztejn, W.H., Drouva, S.V., Pattou, E., Kordon, C. (1978). Effect of morphine on the basal and dopamine-induced release of LHRH from mediobasal hypothalamic fragments in vitro. Eur. J. Pharmacol. 50:285-288.

Samson, W.K,. Said, S.I., Snyder, G., McCann, S.M. (1980). In vitro stimulation of prolactin release by Vasoactive Intestinal Peptide. Peptides, 1:325.

Samson, W.K., Lumpkin, M.D., McCann, S.M. (1984). Presence and possible site of action of secretin in the rat pituitary and hypothalamus. Life Sci., 34:155.

Samson, W.K., Michael, D.L., McCann, S. (1986). Evidence for a physiological role of oxytocin in the control of prolactin secretion. Endocrinology., 119:554-560.

Sarkar, D.K., Fink, G. (1980). Effects of gonadal steroids on output of luteinizing hormone releasing factor into pituitary stalk blood in the female rat. J. Endocrinol. 80:303.

Sarkar, D., Yen, S.S.C. (1985) Hyperprolactinaemia decreases the luteinizing-hormone-releasing-hormone concentration in pituitary portal plasma. A possible role of beta-endorphine as a mediator. Endocrinology., 116:2080-2084.

Schallenberger, E., Oertere, U., Hutterer, G. (1982a). Neuroendocrine regulation of post-partum function. In: Factors influencing fertility in the post partum cow. Ed. by H. Karg and E. Schallenberger. Martinus Nijhoff. Hague. pp. 123-147.

Schallenberger, E., Hutterer, G. (1982b). Frequent pulsatile LH secretion is a prerequisite for a stimulatory feedback action of oestradiol and the reestablishment of oestrous cycles in post partum dairy cows. Acta Endocrinol., 99, 246:16 (Abstr. 19).

Schally, A.V., Arimura, A., Baba, Y., Nair, R.M.G., Matsuo, H. (1971). Isolation and properties of the FSH and LH releasing hormone. Biochem. Biophys. Res. Comm., 43:393-399.

Schally, A.V., Redding, T.W., Arimura, A., Dupont, A., Linthicum, G.L.(1977). Isolation of gamma-aminobutyric acid from pig hypothalami and demonstration of its prolactin release-inhibiting (PIF) activity in vivo and in vitro. Endocrinology., 100:681-691.

Schillo, K.K. (1993). Possible roles of endogenous opioids in control of luteinizing hormone secretion in female ruminants. In: Opioids in farm animals. Ed., N.Parvizi, Landwirtschaftsverlag GmbH, 4400 Münster-Hiltrup, Germany. pp. 1-14

Schofield, J.G. (1983). Used of a trapped fluorescent indicator to demonstrate effects of thyroliberin and dopamine on cytoplasmic calcium concentrations in bovine anterior pituitary cells. FEBS, Lets., 159,79.

Schoors, D.F., Vauquelin, G.P., De Vos, H., Smet, G., Velkeiner, B., Vanhaaelst, L., Dupont, A.G. (1991). Identification of a D1 receptor, not linked to adenylate cyclase, on lactotroph cells. Br. J. Pharmacol. 103:1928-1934.

Selmanoff, M., Wise, P.M. (1981). Decreased dopamine turnover in the median eminence in response to suckling in the lactating rat. Brain Res., 212:101-115.

Selmanoff, M. Selmanoff, C. (1983). Role of pup, age, estradiol-17beta and pituitary responsiveness in the differences in the suckling induced prolactin response during early and late lactation. Biol. Reprod., 29:400-411.

Sesti, L.A., Britt, J.H. (1993). Influence of stage of lactation, exogenous LHRH, and suckling on estrus, positive feed-back of LH, and ovulation in sows treated with estrogen. J. Anim. Sci., 71:989-998.

Sharp, B., Karson, B., Marrshak, D., Ross, R., George, R., Sowers, J., Yamada, T. (1982). Domperidone elevates rat plasma ß-endorphin-immunoreactivity when administered peripherally but not intracerebroventricularly. Life Sci. 31:981-985.

Sharp, D.C., Grumbach, W.R., and Wasserman, C., (1985). Effect of naloxone on gonadotrophin releasing hormone (GnRH) administration to anoestrous mares. Biol. Reprod., 32: (suppl.1), abstr. 153.

Sharpe, P.J., Fraser, H.M. (1978). The endocrine hypothalamus. In: Control of Reproduction. Ed. S.L. Jeffcoate and T.S.M. Hutchinson. Academic Press, London, pp. 271-332.

Shaw, H.J., Foxcroft, G.R. (1985). Relationship between LH, FSH, and prolactin secretion and reproductive activity in the weaned sow. J. Reprod. Fertil., 75:17-28.

Sherey, M.P., Clark, H.J., Franks, S. (1986). The dopaminergic regulation of 45CA2+ homeostasis and prolactin secretion. J. Endocrinol., 108;423.

Sheward, W.J., Fraser, H.M., Fink, G. (1985). Effect of immunoneutralization of thyroptrophin-releasing hormone on the release of thyrotrophin and prolactin during suckling or in response to electrical stimulation of the hypothalamus in anaesthetized rats. J. Endocrinol., 106:113-119.

Shin, S.H., Reifel, C.W. (1981). Adenohypophysis has an inherent property for pulsatile prolactin secretion. Neuroendocrinol., 32:139-144.

Shina, Y.N., Gilligan, T.A. (1981) Identification of a immunoreactive form of prolactin in rat pituitary. Endocrinology.,108(3):1091-1094.

Short, R.E., Randal, R.D., Staigmiller, R.B., Bellows, R.A. (1979). Factors affecting estrogen-induced LH release in the cow. Biol. Reprod., 21:683-689.

Short, R.E., Brooks, A.N., Peters, A.R. & Lamming, G.E. (1987) Opioid modulation of LH secretion during the oestrous cycle of heifers. J. Reprod. Fertil. 80,213-219.

Silverman, A.J., Krey, L.C., Zimmerman, E.A. (1979). A comparative study of the luteinizing hormone releasing hormone (LH-RH) neuronal networks in mammals. Biol. Reprod., 20:98-110.

Sirinathsinghji, D.J., Martini, L. (1984). Effects of bromocriptine and naloxone on plasma level of prolactin, LH and FSH during suckling in the female rat: response to gonadotrophin releasing hormone. J. Endocrinol., 100:175-182

Smith, M.F., Walters, D.L., Harms, P.G., Wiltbank, J.N. (1977). Luteinizing hormone levels after steroid and or 48 hours of calf removal in anestrous cows. J. Anim. Sci. (suppl. 1), 45:209.

Smith, M.S. (1978). The relative contribution of suckling and prolactin to the inhibition of gonadotropin secretion during lactation in the rat. Biol. of Reprod., 19:77-83.

Smith, B.B., Wagner, W.C. (1985). Effect of dopamine agonist or antagonist,TRH, stress and piglet removal on plasma prolactin concentrations in lactating gilts. Theriogenology, 23:283-296.

Sneider, H.P.G., McCann, S.M. (1970). Mono- and indolamines and control of LH secretion. Endocrinology., 86:1127-1133.

Sokoloff, P., Giros, B., Matres, M.P., Bouthenet, M.L., Schwartz, J.C. (1990). Molecular cloning and characterization of novel dopamine receptor (D3) as a target for neuroleptics. Nature, 347:146-151.

Spampinato, S., Locatelli, V., Cpcchi, D., Vicentini, L., Bujusz, S., Ferri, S., Muller, E.E. (1979). Involvement of brain serotonin in the prolactin releasing effect of opioid peptides. Endocrinology., 105:163-166.

Spiegel, K., Kourides, I.A., Pasternak, G.V. (1982). Prolactin and growth hormone release by morphine in the rat: different receptor mechanisms. Science, 217:744-747.

Sewannyana, E., Lincoln, G.A. (1990). Regulation of the photopheriod-induced cycle in the peripheral blood concentrations of beta-endorphin and prolactin in the ram: role of dopamine and endogenous opioids. J. Endocrinol., 127:461-469.

Stanley, H.F., Curtis, A.Y., Sheward, W.J., Roberts, J.L., Fink, G. (1986). Prolactin messenger ribonucleic acid levels in the normal and hypogonadal mouse pituitary gland. Endocrinology., 119:2422-2426.

Steger, R.W., Matt, K.S., Klemcke, H.G., Bartke, A. (1985). Interactions of photoperiod and ectopic pituitary grafts on hypothalamic and pituitary function in male hamsters. Neuroendocrinol., 41:89-96.

Stevenson, J.S., Britt, J.H. (1981a). Luteinizing hormone, total estrogens and progesterone secretion during lactation and after weaning in sows. Theriogenology, 14:453-462.

Stevenson, J.S, Cox, N.M., Britt, J.H. (1981b). Role of the ovary in controlling luteinizing hormone, follicle stimulating hormone and prolactin secretion during and after lactation in pigs. Biol. Reprod., 24:341-353

Stevenson, J.S., Britt, J.H. (1981c). Interval from weaning to oestrus in the sow and performance of pigs after alteration of litter size during late lactation. J. Anim. Sci., 53:1, 177-181.(suppl. 1), 393.

Stewart, J.K., Clifton, D.K., Koerker, D.J., Rogol, A.D., Jaffe, T., Goodner, C.J. (1985). Pulsatile release of growth hormone and prolactin from the primate pituitary in vitro. Endocrinology., 116:1-5.

Sunahara, R.K., Guan, H., O'Dowd, B.F., Seeman, P., Laurier, L.G., George, S.R., Torchia, J., Van Tol, H.h., Niznik, H.B. (1991). Cloning of gene for human dopamine D5 receptor with higher affinity for dopamine than D1. Nature, 350:614-619.

Talwalker, P.K., Ratner, A., Meites, J. (1963). In vitro inhibition of pituitary prolactin synthesis and release by hypothalamic extract. Am. J. Physiol., 205:213-218.

Tamanini, C., Crowder, M.E., Nett, T.M. (1986). Effect of oestradiol and progesterone on pulsatile secretion of LH in ovariectomized ewes. Acta Endocrinol., 111, 122-128.

Tanaka, T., Aiba, T., Shishiba, Y. (1984). The heterogeneity of serum hPRL in a patient with giant prolactinoma. Folia Endocrinol. Jap., 60:1058.

Tang, L.K.L., Spies, H.G. (1975). Effect of gonadal steroids on basal and LHRH induced gonadotropin secretion by cultures of rat pituitary. Endocrinology. 96:349-56

Tashjian, A.H., Barowsky, N.J., Jensen, D.K. (1971). Thyrotropin releasing hormone: direct evidence for stimulation of prolactin production by pituitary cells in culture. Biochem. Biophys. Res. Commun., 43:516-523.

Thomas, G.B., Pearse, D.T., Oldham, C.M., Martin, G.B., Lindsay, D.R. (1988). Effects of breed, ovarian steroids and season on the pulsatile secretion of LH in ovariectomized ewes, J. Reprod. Fertil., 84:313-324.

Thomas, G.B., Cummins, J.T., Doughton, B.W., Griffin, N., Smythe, G.A., Glesson, R.M., Clarke, I.J. (1989). Direct pituitary inhibition of prolactin secretion by dopamine and noradrenaline in sheep. J. Endocrinol., 123:393-402.

Threlfall, W.R., Dale, H.E., Martin, C.E. (1974). Porcine blood and hypophyseal prolactin values. Am. J. Vet. Res., 35:1491-1494.

Tolis, G., Hickey, J., Guyda, H. (1975). Effect of morphine on serum growth hormone, cortisol, prolactin and thyroid stimulating hormone in woman. J. Clin. Endocrinol. Metab., 41:797-805.

Tokach, M.D., Pettigrew, J.E., Dial, G.D., Wheaton, J.E., Crooker, B.A., Johnston, L.J. (1992). Characterization of LH secretion in the primiparous lactating sow:relationship to blood metabolites and return to estrus interval. J. Anim. Sci. 70:2195-2201.

Thorner, M., Besser, G.M., Jone, A., Dacie, J., Jones, A.E. (1975). Bromocriptine therapy of female infertility - a report of 13 pregnancies. Br. Med. J., 4:649-651.

Trout, W.E., and Malven, P.V., (1987). Effects of exogenous oestradiol-17β and progesterone on naloxone-reversible inhibition of the release of luteinizing hormone in ewes. J. Anim. Sci., 65:1602-1609.

Trudeau, V.L., Pharazyn, A., Beltranena, E., Aherne, F.X. (1988a). Naloxone elevates plasma follicle stimulating hormone but not luteinizing hormone levels in the immature male pig. Can. J. Anim. Sci., 69:1095-1098.

Trudeau, V.L., Meijer, J.C., van de Wiel, D.F.M., Bevers, M.M. and Erkens, J.H.F. (1988b). Effects of morphine and naloxone on plasma levels of LH, FSH, prolactin and growth hormone in the immature male pig. J. Endocrinol., 119:501-508.

Tsai-Morris, C.H., Chosh, M., Hirshfield, A.N., Wise, P.M. Brodie, A.M.H. (1983). Inhibition of ovarian aromatase by prolactin in vivo. Biol. Reprod. 29:342-346.

Tucker, H.A., (1988). Lactation and its hormonal control. In: The Physiology of Reproduction. Eds. E. Knobil, J.Neill, Raven Press; New York. pp2235-2263.

Tyson, J.E. (1977). Nursing and prolactin secretion: principal determinants in the mediator of puerperal infertility. In: Prolactin and human reproduction. Ed. by P.G. Crosignani, C. Robyn. Academic Press, London, pp. 97-108.

Uemura, H., Kobayashi, H. (1971). Effects of dopamine implanted in the median eminence on the oestrus cycle in the rat. Endocrinol. Jap., 18:91-94.

Unterwald, E.M., Horme-King, J., Kreek, M.J. (1992). Chronic cocaine alters brain mu opioid receptors. Brain Research, 582(1-2) 314-318.

Urbanski, H.R., Pickle, R.L., Ramirez, V.D. (1988). Simultaneous measurement of gonadotrophin-releasing hormone, luteinizing hormone and follicle stimulating hormone in the orchidectomized rat. Endocrinology., 123:413-419.

Vaitukatis, J.L., Ross, G.T., Braustein, G.A., Rayford, P.L. (1976). Gonadotropins and their subunits; basic and clinical studies. Rec. Prog. Horm. Res., 32:289- 332.

Vale, W., Rivier, J., Vaughan, J., McClintock, R., Corrigan, A., Woo, W., Carr, D., Spiess, J. (1986). Purification and characterization of an FSH releasing protein from porcine ovarian follicular fluid. Nature, Lond., 321:776-779.

Van de Wiel, D.F.M., Van Landeghem A.A.J., Bevers, M.M., Willemse, A.H. (1978). Endocrine control of pituitary and ovarian function during lactation in the sow. In: Comparative Endocrinol. Eds. P.J.

Gaillard, H.H. Boer. Elsevier/North Holland Press, pp. 372-378.

Van de Wiel, D.F.M., Booman, P., Willemse, A.H., Bevers, M.M. (1985). Relevance of prolactin to lactational and post-weaning anoestrus in the pig. In: Endocrine causes of seasonal and lactational anoestrus in farm animals. Eds. F. Ellendorf and F. Fisher, Martinus-Nijhoff Pub. pp. 154-164.

Van Landeghem, A.A.J., Van de Wiel, D.F.M. (1978). Radioimmunoassay for porcine prolactin plasma levels during lactation, suckling and weaning and after TRH administration. Acta Endocrinol., 88:(4), 653-667.

Van Vugt, D.A., Meites, J. (1980) Influence of endogenous opiates on anterior pituitary function. Fed. Proc., 39:2533-2538.

Van Vugt, D.A., Bakst, G., Dyrenfurth, I., Ferin, M. (1983). Naloxone stimulation of LH secretion in female monkeys: influence of endocrine and experimental conditions. Endocrinology., 1134:1858-1864.

Van Vugt, D.A., Sylvester, P.W., Aaylsworth, C.F., Meites, J. (1982). Cointeraction of gonadal steroid inhibition of LH release by naloxone. Neuroendocrinol., 34:274- 278.

Van Landeghem, A.A.J., Van de Wiel, D.F.M. (1978). Radioimmunoassay for porcine prolactin plasma levels during lactation, suckling and weaning and after TRH administration. Acta Endocrinol., 88:(4), 653-667.

Varley, M.A., Atkinson, T. (1985). Weaning at birth: the effect on reproduction of the sow. Anim. Prod., 41:375-382.

Variey, M.A., Foxcroft, G.R. (1990). Endocrinology of lactation. J. Reprod. Fert., 40:47-61.

Veldhuis, J.D., Klase, P., Hammond, J.M.(1980a). Divergent effect of PRL upon steroidogenesis by porcine granulosa cells in vitro: influence of cytodifferentiation. Endocrinology., 107:42-46.

Vermes, I., Tilders, F.J.H., Maysinger, (1984). Release of opioid peptides from rat hypothalamus in vitro: dopamine-receptor mediated inhibition of beta-endorphine release. In: Central and peripheral endorphins: Basic and clinical aspects. Eds. E.E. Muller, A.R. Genazzani, Raven Press, New York. pp. 109-113.

Voogt, J.L., Carr, L.A. (1974). Plasma prolactin levels and hypothalamic catecholamine synthesis during suckling. Neuroendocrinol., 16:108-118.

Walters, D.L., Kaltenbach, C.C., Dunn, T.G., Short, R.E. (1982a). Pituitary and ovarian function in post partum beef cows. I) Effect of suckling on serum and follicular fluid hormones and follicular gonadotropin receptors. Biol. Reprod., 26:640-646.

Walters, D.L., Short, R.E., Convey, E.M., Staigmiller, R.B., Dunn, T.G., Kaltenbach, C.C. (1982b). Pituitary and ovarian function in post partum beef cows. II) Endocrine changes prior to ovulation in suckled and non-suckled post partum cows compared to cycling cows. Biol. Reprod., 26:647-654.

Walton, J.S., McNeilly, J.R, McNeilly, A.S., Cunningham, F.J. (1977). Changes in blood levels of prolactin, LH. FSH and progesterone during anestrus in the ewe. J. Endocrinol., 75:127-136.

Wang, C., Hsueh, A.J.W., Erickson, G.F. (1980). Prolactin inhibition of estrogen production by cultured rat granulosa cells. Mol. Cell. Endocrinol., 20:135-144.

Wardlaw, S.L., Thoron, L., Franz, A.G. (1982). Effect of sex steroid on brain β-endorphin. Brain Research, 245:327-331.

Wardlaw, S.H., Frantz, A.G. (1983). Brain beta-endorphin during pregnancy, parturition and post-partum period. Endocrinology., 113:1664-1668

Watson, E.D. (1987). Concentrations of LH and oestradiol-17-β in plasma of the dairy cow during the post partum acyclic period. Anim. Prod., 45:1-8.

Webb, R., Lamming, G.E., Haynes, N.B., Hafs, H.D., Manns, J.G. (1977). Response of cyclic and post partum suckled cows to injections of synthetic LH-RH. J. Reprod. Fertil., 50:203-210.

Webb, R., Lamming, G.E. (1981). Pattern of plasma prolactin in post-partum suckled cow. J. Endocrinol., 90:391-396.

Webb, R., Baxter, G., Preece, R.D., Land, R.B., Springbett, A.J. (1985). Control of gonadotrophin release in Scottish Blackface and Finnish Landrace ewes during seasonal anestrus. J. Reprod. Fertil. 73:369-378.

Weitz-Carrington, P., Roux, M.E., McWilliams, Phillips-Quagliata, J.M., Lamm, M.E. (1978). Proc. Natl. Acad. Sci. USA., 75:2928-2932.

Wehrenberg, W.B., Wardlaw, S.L., Frantz, A.G., Ferin, M. (1982). β-Endorphin in hypophysial portal blood: variations throughout the menstrual cycle. Endocrinology., 111:879-881.

Weiner, R.I., Ganong, W.F. (1978). Role of brain monoamines and histamine in regulation of anterior pituitary secretion. Physiol. Res., 58:905-976.

Weisner, J.B., Koening, J.I., Krulich, L., Moss, R.L. (1985). Possible delta receptor mediation of the effect of beta-endorphin in LH release, but not on prolactin release in the ovariectomized rat. Endocrinology., 116:475.

Wheeler, M.B., Anderson, G.B., Munro, C.J., Stabenfeldt, G.H. (1982). Prolactin response in beef cows and heifers suckling one or two calves. J. Reprod. Fertil., 64:243-249.

Whisnant, C.S. and Goodman, R.L. (1988). Effects of an opioid antagonist on pulsatile luteinizing hormone secretion in the ewe vary with changes in steroid negative feedback. Biol. Reprod., 39:1032-1038.

Whisnant, C.S., Kiser, R.E., Thompson, F.N. and Barb, C.R. (1986a). Naloxone infusion increases pulsatile luteinizing hormone release in postpartum beef cows. Dom. Anim. Endocrinol. 3, 49-54.

Whisnant, C.S., Kiser, R.E., Thompson, F.N. and Barb, C.R. (1986b). Opioid inhibition of LH secretion during the postpartum period in suckled beef cows. J. Anim. Sci. 63, 1445-1448.

Whisnant, C.S., Kiser, R.E., Thompson, F.N. and Barb, C.R. (1986c). Influence of calf removal on the serum luteinizing hormone response to naloxone in the postpartum beef cow. J. Anim. Sci. 63 (suppl.), 561.

Wildt, L., Marshall, G., Knobil, E. (1980). Experimental induction of puberty in the infantile female rhesus monkey. Science, N.Y., 202:1373-1375.

Williams, G.L., Ray, D.E. (1980). Hormonal and reproductive profiles of early post partum beef heifers after prolactin suppression or steroid-induced luteal function. J. Anim. Sci., 50:906-918.

Wilson, M.E., (1993). Oestradiol negative feedback inhibition on LH secretion during lactation is prolonged in adolescent primiparous rhesus monkeys. J. Endocrinol., 136:127-136.

Winiger, B.P., Wuarin, F., Zahnd, G.R., Wollehim, C.B., Schlegel, W. (1987). Single cell monitoring cytosolic calcium reveals subtype of rat lactotrophs with distinct responses to dopamine and thyrotropin-releasing hormone, Endocrinology., 121:2222-2228.

Witkin, J., Paden, C., Silverman, A. (1982). The luteinizing hormone-releasing hormone (LHRH) systems in the rat brain. Neuroendocrinol., 35:429-438.

Wolinska, E., Polkowska. J., Domanski, E. (1977). The hypothalamic centres involved in the control of production and release of prolactin in sheep. J. Endocrinol., 73:21-29.

Wright, P.J., Jenkin, G., Heap, R.B. (1981a). Prolactin and LH release in response to LH-RH and TRH in ewes during dioestrus,pregnancy and post partum. J. Reprod. Fertil., 62:447-453.

Wright, P.J., Geytenbeek, P.E., Clarke, I.J., Findlay, J.K. (1981b). Evidence for a change in oestradiol negative feed back and LH pulsatile frequency in post partum ewes. J. Reprod. Fertil., 63:97-102.

Yamaji, T., Dierschke, D.J., Bhattacharya, A.N., Knobil, E. (1972). The negative feedback control by estradiol and progesterone of LH secretion in the ovariectomized rhesus monkey. Endocrinology., 90:771-777.

Yang, K., Haynes, N.B., Lamming, G.E. and Brooks, A.N. (1988). Ovarian steroid hormone involvement in endogenous opioid modulation of LH secretion in mature ewes during the breeding and non-breeding seasons. J. Reprod. Fertil., 83:129-139.

Yen, S.S.C., Vanderberger, G., Rebar, R., Ehara, Y. (1972). Variation of pituitary responsiveness to synthetic LHRF during different phases of the menstrual cycle. J. Clin. Endocrinol. Metab., 35:931-934.

Ziecik, A., Tilton, J.E., Weigl, R., Williams, G.L. (1982/83). Plasma luteinizing hormone during pregnancy in the pig. Anim. Reprod. Sci., 5:213-218.

Zukin, R.S, Zukin, S.R. (1981). Multiple opiate receptors. Emerging concepts. Life Sci. 29:2681-2693.

# CHAPTER 2

## EXPERIMENT 1: LH AND PROLACTIN RESPONSES TO NALOXONE VARY WITH STAGE OF LACTATION IN THE SOW.

## 2.1 INTRODUCTION

Acyclicity in the post-partum period in the sow is generally thought to be attributable to inhibitory inputs to the GnRH pulse generator, suppressing pulsatile LH release, follicular development and steroidogenesis (for review see Britt et al., 1985; Varley and Foxcroft, 1990). This interpretation is consistent with our observations of a distinct increase in episodic type LH secretion in a proportion of sows in the immediate post-partum period (De Rensis et al., 1993). Indirect evidence that suppression of the GnRH pulse generator is the primary block to LH secretion comes from studies of LH responsiveness to a single GnRH challenge (Rojanasthien et al., 1987) and to repeated GnRH injections during lactation (Bevers et al., 1981; Britt et al., 1985). Evidence supporting a role for endogenous opiates in the suppression of GnRH post-partum and during lactation has accumulated in pigs, sheep and cattle (for review see Haynes et al., 1989; Barb et al., 1991).

While the opioidergic system appears to play a well defined inhibitory role in the post-partum regulation of LH, evidence for opioidergic control of FSH secretion is contradictory. Opioids have been shown to inhibit (Pang et al., 1977), stimulate (Bhanot et al., 1984) or have no effect (Ieiri et al., 1980; Dondi et al., 1991) on FSH secretion. In young male pigs morphine suppressed, whereas naloxone increased FSH levels (Trudeau et al., 1988a, b) and in lactating sows (Barb et al., 1987) naloxone stimulated FSH secretion. During lactation the blockade of opiate receptors with naloxone results in a decrease of prolactin secretion in the rat (Sirinathsinghji et al., 1984), sheep (Gregg et al., 1986) and sow (Mattioli et al., 1986; Barb et al., 1986; Armstrong et al., 1988). In a previous study we observed that inhibition of LH secretion only becomes established in suckled sows around 66-78h post-partum (De Rensis et al., 1993). In sows weaned at birth, LH secretion is maintained beyond this time and is associated with immediate follicular development.

Therefore the aim of the present experiment was to use the opioid receptor antagonist naloxone to study the involvement of endogenous opioids in the development of inhibitory control of LH secretion and follicular development, and associated effects on FSH and PRL secretion during early lactation in the sow. A preliminary dose response experiment was carried out to establish an effective dose of naloxone for use in the early post-partum period.

## 2.2 MATERIALS AND METHODS

### 2.2.1 Animals and blood collection

A total of 17 multiparous crossbred sows from the University of Alberta herd were used. Sows were housed in conventional farrowing crates and water and feed were provided ad libitum throughout lactation. The average weight of the sows one day after farrowing was 207.0±8.3kg and the average litter size during the experiment was 10.7±0.7 piglets.

The preliminary experiment (Experiment 1A) was used to confirm that doses of naloxone effective in increasing LH at later stages of lactation (Mattioli et al., 1986; Barb et al., 1986; Armstrong et al.,1988) were also effective in the early post-partum period. Six sows received jugular catheters 12-18h post-partum under general anesthesia and were continuously sampled at 15-min intervals from 66 to 78h post-partum. Three sows received an i.v. injection of 4mg/kg naloxone hydrochloride (Sigma Chemical Co., St. Louis, MO) 6h after the beginning of sampling (72h after farrowing) and a second injection of 2mg/kg three hours later (75h after farrowing). The remaining three sows received similar treatments in the reverse order. As a positive control, all sows were blood sampled at 15-min intervals for 3h before and 3h after administration of 2mg/kg naloxone hydrochloride, at day 10 post-partum.

In the main experiment (Experiment 1B), eleven sows were used to investigate the effect of chronic naloxone treatment in the early post-partum period. Sows received indwelling jugular catheters under general anesthesia either two days before the expected day of farrowing (8 sows) or 12-18h after parturition in the remaining nine animals. This change in protocol was adopted to avoid extensive periods between cannulation and the time of first sampling. Blood samples (2.5 ml) were collected at 15-min intervals over five periods: from 12 to 18h, 36 to 48h, 54 to 60h and 72 to 78h post-partum. Six sows (Naloxone group) received initial i.v. injections of 2 mg/kg of naloxone hydrochloride at 39h post-partum in sterile physiological saline, followed by 13 further injections of 1mg/kg at 3h intervals from 42 to 78h post-partum. The rationale for this treatment regimen has been previously described by Cosgrove et al. (1991). Control sows received saline vehicle only. In three sows (one control and two treated), which were cannulated after farrowing to reduce the interval between cannulation and start of blood sampling, no samples were taken during the first period (12 to 18h post-partum).

To relate treatment induced changes in LH secretion to follicular development, ovarian tissue was recovered by laparotomy from 4/6 naloxone and 4/5 control sows 9.2±0.7h after final naloxone injection (90-96h after parturition). Follicular development was assessed by estimating follicular diameter (FFD) of the ten largest follicles on each ovary on the basis of follicular fluid volume, using the relationship established in previous studies of pig follicles by Grant et al. (1989). As a welfare consideration and in the absence of treatment-induced effects on follicular characteristics, the remaining three sows were not ovariectomized.

Irrespective of ovarian status, all sows were also blood sampled at 15 min intervals for a six-hour period on day 10 of lactation. Three hours after the start of sampling, all sows were treated with a single injection of 2mg/kg naloxone hydrochloride as a positive control.

### 2.2.2 Hormone assays

Plasma LH was quantified in all samples. Plasma PRL was assayed in all samples in Experiment 1A. In Experiment 1B, PRL was analyzed in samples taken every 15 min for the second and last period and every 30 min during other periods. Plasma FSH was measured at hourly intervals.

Plasma LH concentrations were determined using the double antibody RIA described by De Rensis et al. (1993). The inter- and intra-assay coefficients of variation were 4.1% and 12.7%, respectively. The sensitivity of the assay, defined as 90% of total binding, was 0.13ng/ml.

Plasma FSH concentrations were quantified by an homologous double-antibody RIA also described previously by De Rensis et al. (1993). The purified porcine FSH used for reference standard was USDA-pFSH-B-1 kindly supplied by Dr. D.J. Bolt (USDA Animal Hormone Program, Reproduction Laboratory, Beltsville Agricultural Research Centre, Beltsville, Md 20705). The intra- and inter-assay c.v. were 5.9 and 12.8%, respectively and the overall limit of sensitivity of the assay defined as 85% of total binding was 3.5ng/ml.

Plasma concentrations of PRL were measured by the method described by Shaw and Foxcroft (1985) with minor modifications. Intra- and inter-assay c.v. were 3.9 and 10.7%, respectively. The overall sensitivity of the assay defined as 85% of total binding was 1.9 ng/ml.

### 2.2.3 Statistical analysis

In experiment 1, analysis of variance for repeated measures (PROC GLM, SAS statistical package, (1985)) was applied to mean LH data for the initial 6h and subsequent 3h time blocks for each set of sows studied, to establish effects of naloxone treatment (time block). In the event of significant time effects, Student-Newman-Keuls multiple range test was used to compare differences between block means. The same analysis was applied to the data of all sows studied at day 10 post-partum. Additionally, correlation analysis was used to establish overall interrelationships between the doses of naloxone used and the mean LH plasma concentrations.

In the main experiment, plasma concentrations of LH, FSH and PRL were also analyzed by analysis of variance for repeated measures (time block). Within sampling periods, hormone means for consecutive three hour time blocks (from 12 to 78h post-partum and on day 10) were calculated for individual sows and these data were analyzed with treatment, sow and time as discrete (class) variables. Treatment effects (naloxone versus control) were tested against sow within treatment; time and time x treatment interactions were tested using time x sow within treatment as the error term. In the presence of time effects but in the absence of treatment effects, period means were compared by Student's t-test using the combined data. Differences in follicular size were analyzed by analyses of variance with treatment and sow as independent variables. The main effect, treatment, was tested against sow within treatment.

## 2.3 RESULTS

### Plasma LH

Experiment 1A; Administration of both 2 and 4mg/kg of naloxone 72h post-partum increased ($p<0.05$) mean LH levels (Fig. 2.1). A consideration of individual profiles revealed clear responses in 4/6 sows (data not shown). In the remaining animals, the 4mg/kg naloxone dose elicited an LH response in one sow, whereas in the others there was no treatment effect. In four sows, the 4mg/kg dose induced behavioral side effects (lateral head movement, vocalisation, salivation). A positive correlation between the dose of naloxone and mean plasma LH concentrations after treatment was observed ($R=0.734$, $p<0.001$). Treatment with the lower dose of 2mg/kg of naloxone at day 10 of lactation was also effective in increasing LH plasma levels ($p<0.05$) in 4/6 sows. On the basis of these data an initial dose of 2mg/kg of naloxone was considered appropriate for the main experiment.

Experiment 1B; The individual patterns of LH secretion in four naloxone and four control sows are shown in Fig. 2.2 and 2.3 and represent the range in the level of LH secretion observed. Mean LH concentrations in both naloxone and control sows were significantly higher ($p<0.05$) during the first 48h post-partum than in the pre-treatment time block at day 10 (Fig. 2.4). Analysis of pooled data established a significant difference in mean LH concentrations between 12-15h and 75-78h post-partum ($P<0.05$). This decline in LH concentrations was more pronounced by day 10 of lactation (Fig. 2.4). Repeated naloxone injections at three hour intervals from the 39 to 78h post-partum (Fig. 2.4) did not modify LH secretion ($p=0.30$). Moreover, no differences ($p=0.21$) were detected between the pre- and the post-injection LH means at the time of the first naloxone injection 39h post-partum. Conversely, when naloxone was injected on day 10 of lactation, a significant increase in plasma LH was observed ($p<0.05$) in all but three sows (e.g. sow G, Fig. 2.3).

## Plasma FSH

Administration of naloxone 72h post-partum at either 2 or 4mg/kg naloxone did not significantly affect plasma FSH. Three hour mean plasma concentrations during the five periods of sampling in Experiment 1B are shown in Fig. 2.5. Treatment with naloxone did not alter FSH levels either during the first 78h post-partum or at day 10 of lactation. However, in both groups there was a significant ($p<0.05$) increase in plasma FSH levels between the early post-partum period and day 10 of lactation. High variability ($p<0.001$) in FSH levels between sows in all groups was observed.

## Plasma Prolactin

Experiment 1A; Administration of both 2 and 4 mg/kg of naloxone significantly ($p<0.001$) decreased plasma PRL in 4/6 sows. However there was no correlation between the dose of naloxone used and the plasma PRL levels.

Experiment 1B; The individual patterns of PRL secretion in two naloxone and two control sows are shown in Fig. 2.6 and overall mean (+SEM) plasma concentrations of PRL during the five sampling periods are shown in Fig. 2.7. During the first 78h post-partum there was no effect of naloxone on plasma PRL. At day 10 of lactation there was a significant ($p<0.05$) decrease in PRL after naloxone injection (Fig. 2.7) in all but one animal.

## Follicular Development

Ovarian development was assessed in the first eight sows sampled (4 control and 4 treated). There were no differences ($p=0.39$) in estimated follicular diameter between the naloxone and control groups ($3.6\pm0.62$ mm and $4.5\pm0.69$ mm, respectively), indicating that naloxone had no direct or indirect effect on follicular development. Because we did not observe any difference in follicular development between groups at this stage of the experiment and the data confirmed previous studies done by De Rensis et al. (1993) and others (Palmer et al., 1965; Kunavongkrit et al., 1984), animal welfare considerations suggested that further study of follicular development involving surgical ovariectomy of the remaining sows was not warranted.

## 2.4 DISCUSSION

Irrespective of treatment, a gradual suppression of LH secretion with time from farrowing was observed. This decline and the presence in this study of elevated LH plasma levels during the first 48h post-partum compared with day 10 of lactation is in agreement with our previous data in the sow (De Rensis et al., 1993) and recent studies in the rat (Dondi et al., 1991).

The preliminary experiment indicated that naloxone was able to antagonize the inhibitory effect of endogenous opioids on LH secretion 72h after farrowing in a dose-dependent manner. This observation is in agreement with the recent report in the lamb of a dose-dependent elevation in LH secretion following naloxone injection (Ebling et al., 1989). However in lactating sows, Barb et al. (1986) reported a threshold for LH response at day 22 of lactation of 1mg/kg of naloxone. The difference in the results between this and our study could be related to the period of lactation considered and thus to a difference in opioid tone or opiate receptor populations. Overall, our data suggested that the regimen of 2mg/kg naloxone followed by repeated injection of 1mg/kg adopted in experiment 2 would provide effective antagonism of any endogenous opioid activity.

The chronic administration of naloxone at three hour intervals from 39 to 78h post-partum did not change the pattern of LH secretion compared to control sows, whereas consistent responses were observed at day 10 of lactation. One interpretation of these data is that endogenous opiates do not mediate the initial inhibitory effects of suckling in the early post-partum period. An inhibitory opioidergic regulation of LH secretion clearly exists in established lactation, as shown by several earlier studies (Mattioli et al., 1986; Barb et al., 1986; Armstrong et al., 1988). Evidence for differential opioidergic control of LH secretion in the early and later post-partum period have been recently reported from studies in lactating rat (Wu et al.,1992). A second interpretation of our data could be that the lack of a LH response to naloxone could be related to desensitization of opioid receptors due to repeated treatment with this antagonist. This suggestion is consistent with the observed increase in LH secretion in response to a single, bolus injection of 2mg/kg naloxone 66-78h post-partum in the preliminary experiment and is the subject of further investigation. Owens and Cicero (1981) demonstrated that pre-treatment with naloxone reduced naloxone-induced increases in serum LH in the male rat and Ebling and Lincoln (1985) observed that naloxone administration to male sheep at 4h intervals was associated with a decline in the LH response after each injection of naloxone. Furthermore, Knight et al. (1986) did not obtain a significant naloxone induced increase of serum LH in suckled ewes by repetitive administration of naloxone in small doses (4.17 mg/ewe) every 5min for a 75min period. Conversely, Myers et al. (1989) in beef cows and Rawlings et al. (1991) in the ram did not observe any inhibitory effect of repeated naloxone injections when treating the animals at 2h intervals. Therefore, it would be interesting to establish if chronic naloxone treatment to sows in advanced lactation (when there is an active inhibition of LH secretion by the opioidergic system) is able to induce a prolonged increase in LH secretion.

Thirdly, the absence of a response to the first bolus injection of naloxone 39h after farrowing and to repeated injections between 39 and 78h in most sows could result from a high opioid tone and, therefore, a need for a higher dose of naloxone to overcome LH inhibition at this time. In the cow Whisnant et al. (1986), found that higher doses of naloxone are required to disinhibit LH secretion at day 14 post-partum compared to days 28 and 42 and suggested that opioid inhibition is greater in the early post-partum period. The response of sows of the first experiment to naloxone in the same period, however, suggests that this is unlikely.

Finally, our experimental design does not preclude the possibility that additional inhibitory mechanisms are present in the early post-partum period which continue to suppress LH even when opioid inhibition is blocked by naloxone.

Based on visual appraisal of the data from the main experiment, three sows did not exhibit a clear response to naloxone treatment at day 10 of lactation. The presence of some sows that do not respond to naloxone treatment has also been observed in other studies (Armstrong et al., 1986;Barb et al., 1986; Barb et al., 1991; Mattioli et al., 1986). Also in lactating cows Gregg et al. (1986) and Whisnant et al. (1986) observed that some animals do not respond to naloxone and they related the absence of an LH response to the high levels of cortisol in those animals. Thus we cannot exclude the possibility that in some animals the experimental conditions may have antagonized the ability of naloxone to disinhibit the release of LH. However, as discussed above, other factors such as the dose of naloxone or the period of lactation studied may be involved.

Plasma FSH was significantly higher ($p<0.05$) in both experiments at day 10 of lactation compared with the early post-partum period. These data are in agreement with the results of Stevenson et al. (1981), who observed higher FSH levels at day 30 of lactation compared to day 20. However FSH release appeared to have been unaffected by naloxone treatment at either 2mg/kg or 4mg/kg, suggesting that FSH is relatively insensitive to acute changes in opioid activity.

In contrast Barb et al. (1987) reported an increase in FSH secretion after naloxone treatment in lactating sows and Trudeau et al. (1988a, b) demonstrated that intravenous injection of 0.5 mg/kg naloxone did not affect FSH secretion in 4-5 week old male pigs whereas higher doses (1 and 10 mg/kg) were effective, suggesting a dose dependent response for FSH. In adult ewes, naloxone and morphine did not influence FSH secretion (Currie et al., 1987; Peters et al., 1979), whereas the effect of naloxone on FSH secretion was evident in 10 week old lambs (Rawlings et al., 1991).

Plasma prolactin concentrations in this experiment were high soon after farrowing and then declined with time during lactation. Similar changes in PRL secretion have been reported in previous studies in cattle (Peters et al., 1979) and pigs (Stevenson et al., 1981). The significant ($p<0.001$) decline in PRL after naloxone treatment observed at day 10 of lactation in our study was consistent with data from previous studies in lactating sows which established an effect of naloxone injection on plasma PRL levels in sows treated at day 15 (Mattioli et al., 1986), at day 21 (Armstrong et al., 1988) and at day 22

(Barb et al., 1986) post-partum. However, irrespective of the dose or pattern of naloxone treatment, no significant changes (p=0.18) in plasma PRL were observed in the first 78h post-partum. Similarly in ewes, Gregg et al. (1986) observed a decrease in serum concentrations of PRL after naloxone treatment (1.0mg/kg) on day 14 to 26 but not on day 10 post-partum. As for LH however, we suggest that several inhibitory and stimulatory factors may control PRL secretion during lactation (for review see Ben-Jonathan et al., 1989) and an opioid effect may only become evident later in lactation.

Finally, the absence of differences in follicular development between control and naloxone treated sows indicate that chronic naloxone treatment in the early post-partum period is not able to induce follicular development either by modifying gonadotropin secretion or by a local effect on the ovary.

## 2.5 CONCLUSION

The ability of naloxone to counteract the suppression of LH at days 9-10 of lactation confirms the importance of the opioidergic systems in the block of reproductive activity during established lactation in the sow. The presence of pronounced and eventually declining LH secretion in the early post-partum period confirms our previous data (De Rensis et al., 1993) and again indicates that suckling-induced inhibition of LH secretion in the immediate post-partum period is needed to establish lactational anestrus in the sow. The lack of a naloxone effect for at least the first 50-60h post-partum suggests that the mechanism by which the opioidergic systems block LH secretion during lactation may require some time before becoming operative.

Similarly, the observation that PRL secretion during the early post-partum period was not modified by naloxone but that a significant decrease was evident at day 10 of lactation indicates that there are also temporal differences in the effect of endogenous opioids on PRL secretion during lactation. Naloxone did not affect FSH secretion at any stage of lactation in sows, providing evidence for differential regulation of gonadotropin secretion. Finally, treatment did not modify follicular development, indicating the lack of either direct or indirect effects of naloxone on ovarian activity.

**Fig. 2.1** Mean (+SEM) plasma LH concentrations 66-78h after farrowing in the 3h period before, and the 3h periods after, injection of two doses of naloxone in two groups (n=3) of lactating sows in Experiment 1A. H = high (4mg/Kg) dose and L = low (2mg/Kg) dose of naloxone. Means with different superscripts differ ($p<0.05$).

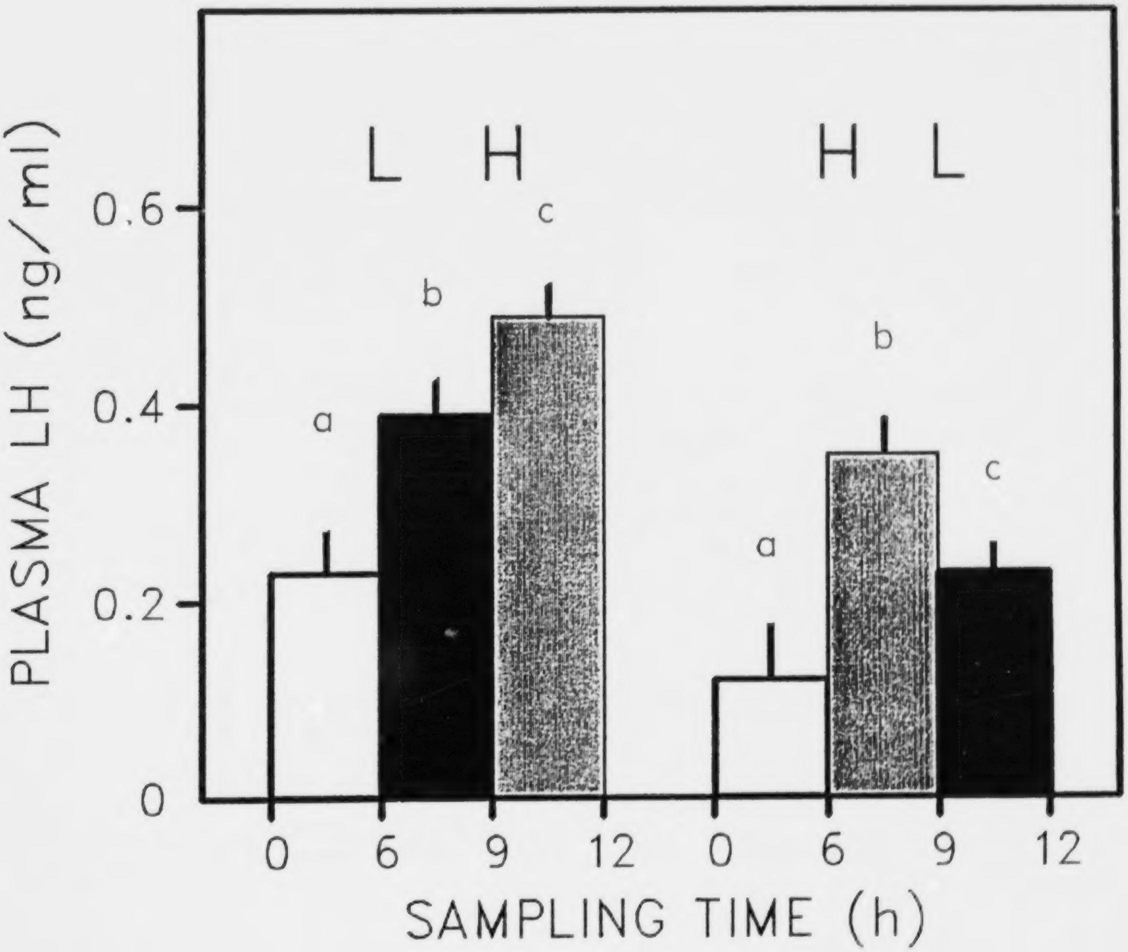

L H
H L
PLASMA LH (ng/ml)
0.6
0.4
0.2
0
a
b
c
a
b
c
0
6
9
12
0
6
9
12
SAMPLING TIME (h)

**Fig. 2.2** Individual plasma LH profiles in four Control sows (C,D,E,L) from Experiment 1B. Arrows indicate time of naloxone injections on day 10 of lactation, at which time sows were either ovariectomized (Ovx) or intact (Int).

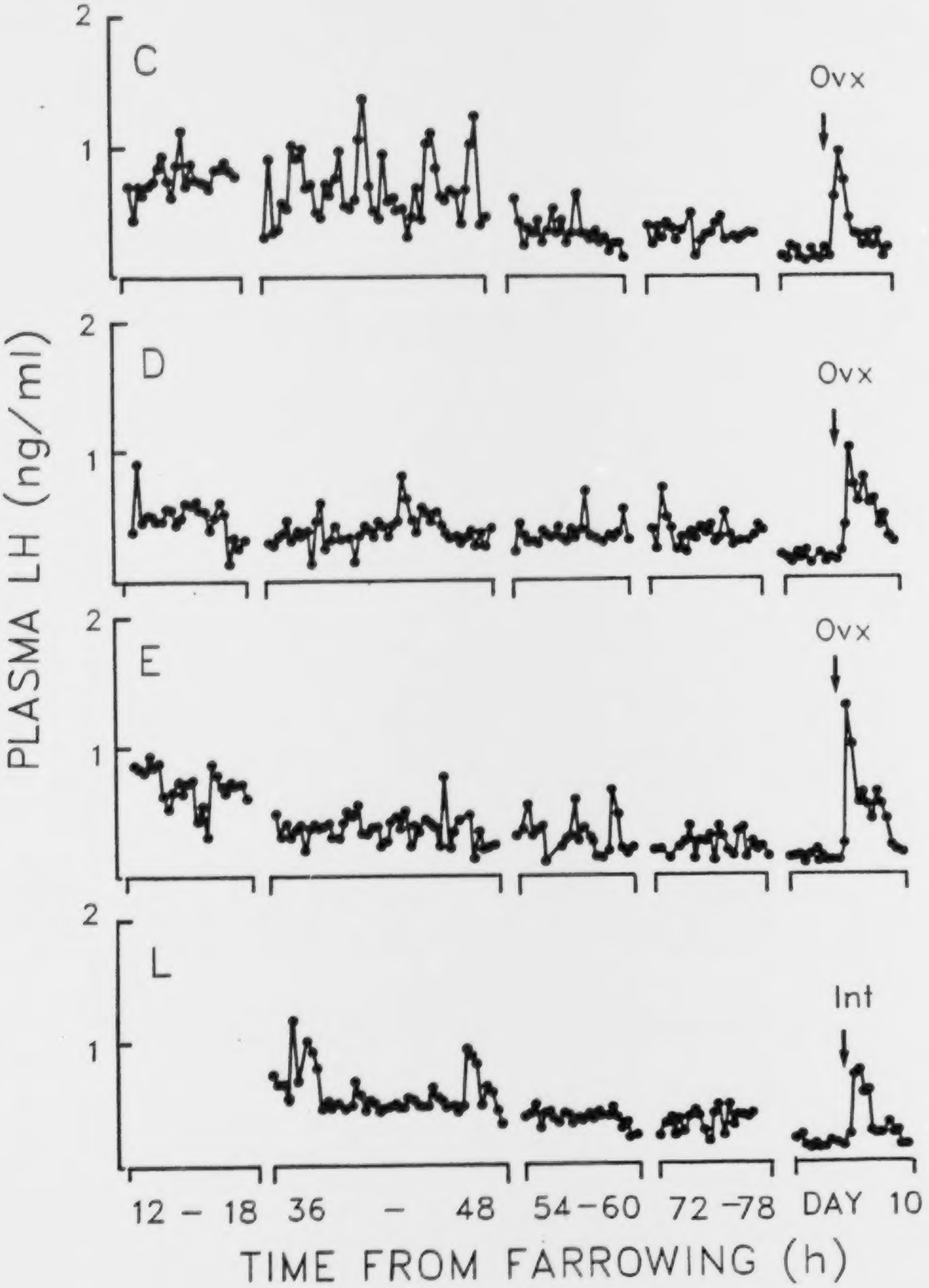

PLASMA LH (ng/ml)
2
1
C
Ovx
2
1
D
Ovx
2
1
E
Ovx
2
1
L
Int
12 - 18
36 - 48
54-60
72 -78
DAY 10
TIME FROM FARROWING (h)

**Fig. 2.3** Individual plasma LH profiles in four Naloxone treated sows (A,B,G,H) from Experiment 1B. Arrows indicate time of naloxone injections during the initial period of sampling and on day 10 of lactation, at which time all these sows were ovariectomized (Ovx).

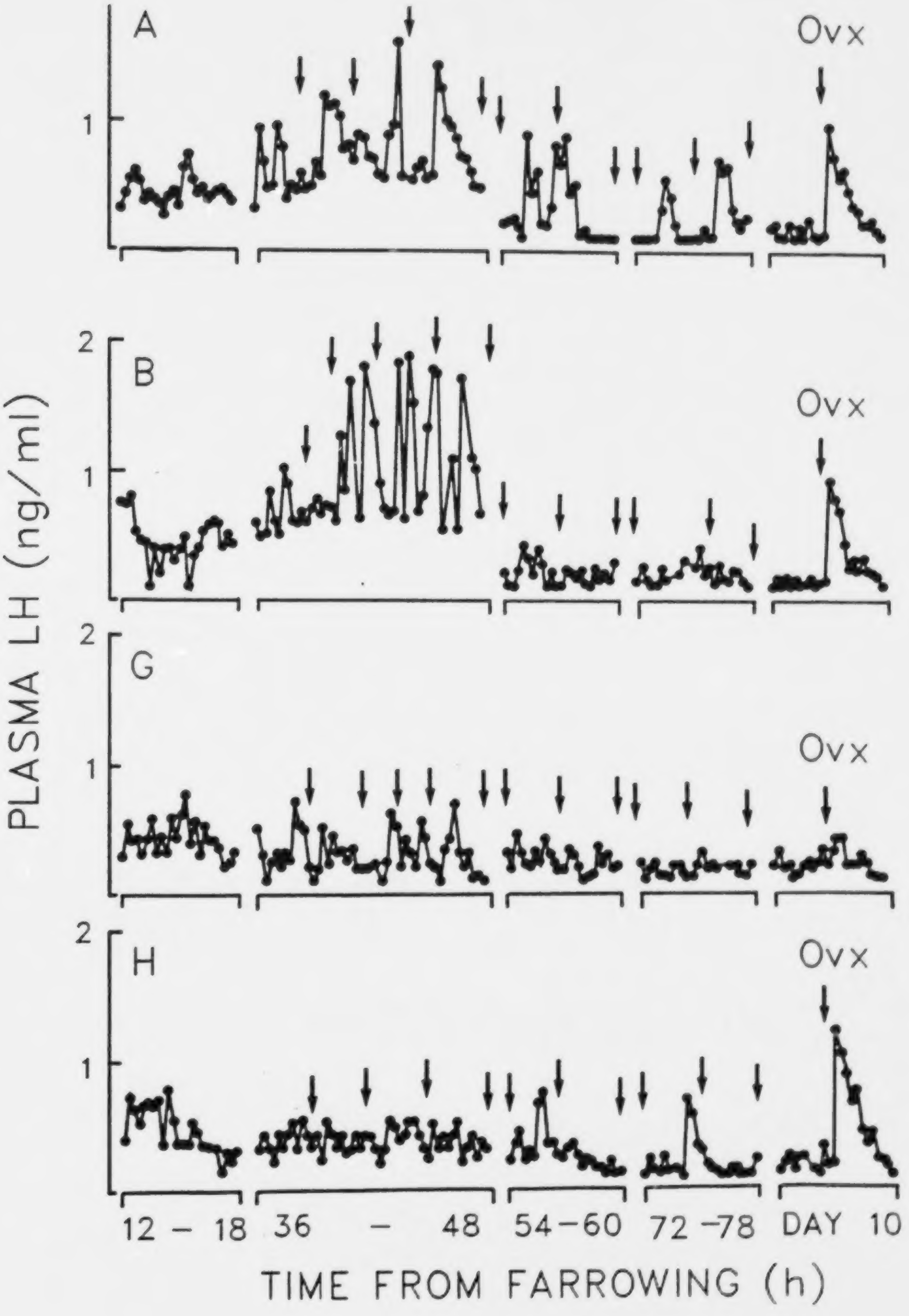

A
Ovx
B
Ovx
G
Ovx
H
Ovx
1
2
PLASMA LH (ng/ml)
12 – 18
36 – 48
54–60
72 –78
DAY 10
TIME FROM FARROWING (h)

**Fig 2.4** Mean (+SEM) plasma LH in Naloxone treated (n=6) and Control sows (n=5) in Experiment 1B. Each bar indicates the means for successive 3h-periods of sampling. Naloxone treatment from 39 to 78h post-partum did not modify LH secretion ($p=0.30$) but analysis of pooled data established a significant difference in mean LH concentrations 12-15h compared to 75-78h post-partum ($P<0.05$). *, significant ($p<0.05$) increase in mean plasma LH concentrations in response to naloxone treatment 3h after the start of sampling on day 10 of lactation.

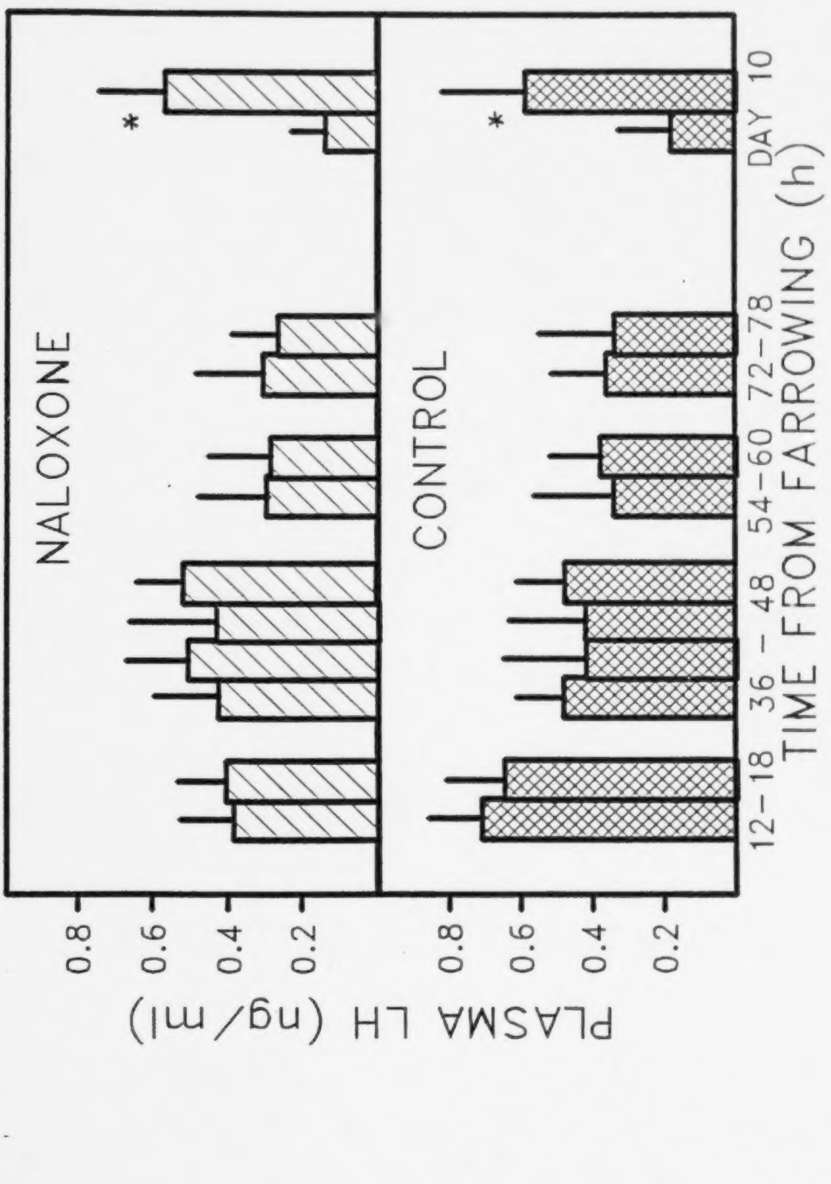

PLASMA LH (ng/ml)
0.8
0.6
0.4
0.2
NALOXONE
CONTROL
*
*
12–18
36 – 48
54–60
72–78
DAY 10
TIME FROM FARROWING (h)

**Fig 2.5** Mean (+SEM) plasma FSH in Naloxone treated (n=6) and Control sows (n=5) in Experiment 1B. Each bar indicates the means for successive 3h-periods of sampling. Treatment with naloxone did not alter plasma FSH either during the first 78h post-partum or at day 10 of lactation. However, in both groups there was a significant ($p<0.05$) increase in plasma FSH between the early post-partum period and day 10 of lactation.

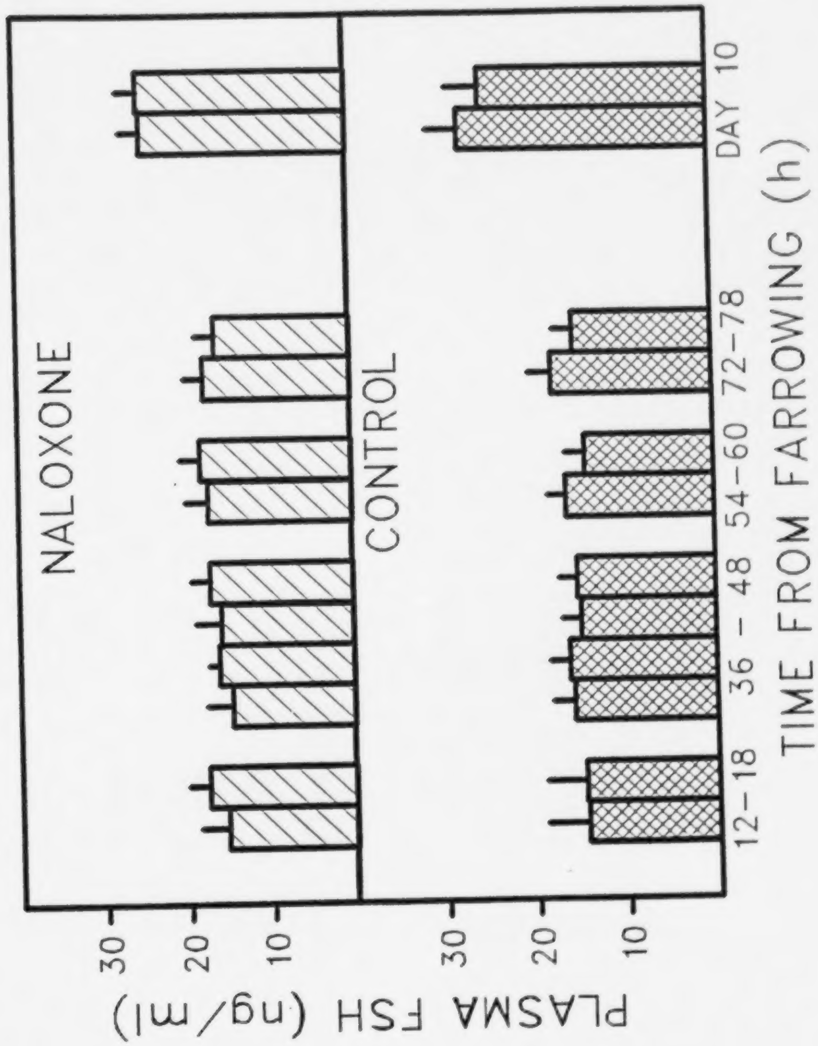

NALOXONE
CONTROL
PLASMA FSH (ng/ml)
30
20
10
30
20
10
12–18
36 – 48
54–60
72–78
DAY 10
TIME FROM FARROWING (h)

**Fig 2.6** Individual plasma prolactin profiles in two Naloxone treated and two Control sows in Experiment 1B. Arrows indicate naloxone injections.

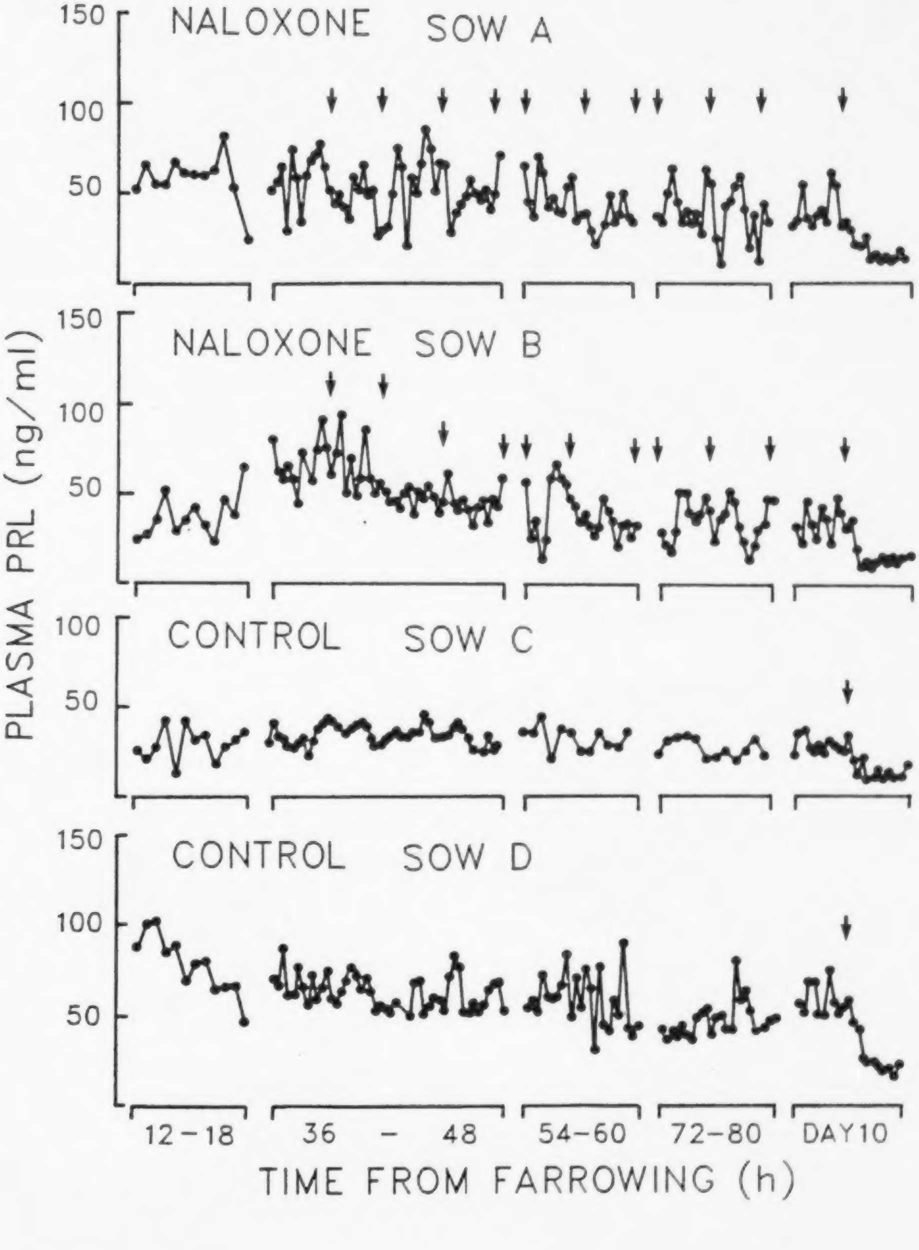
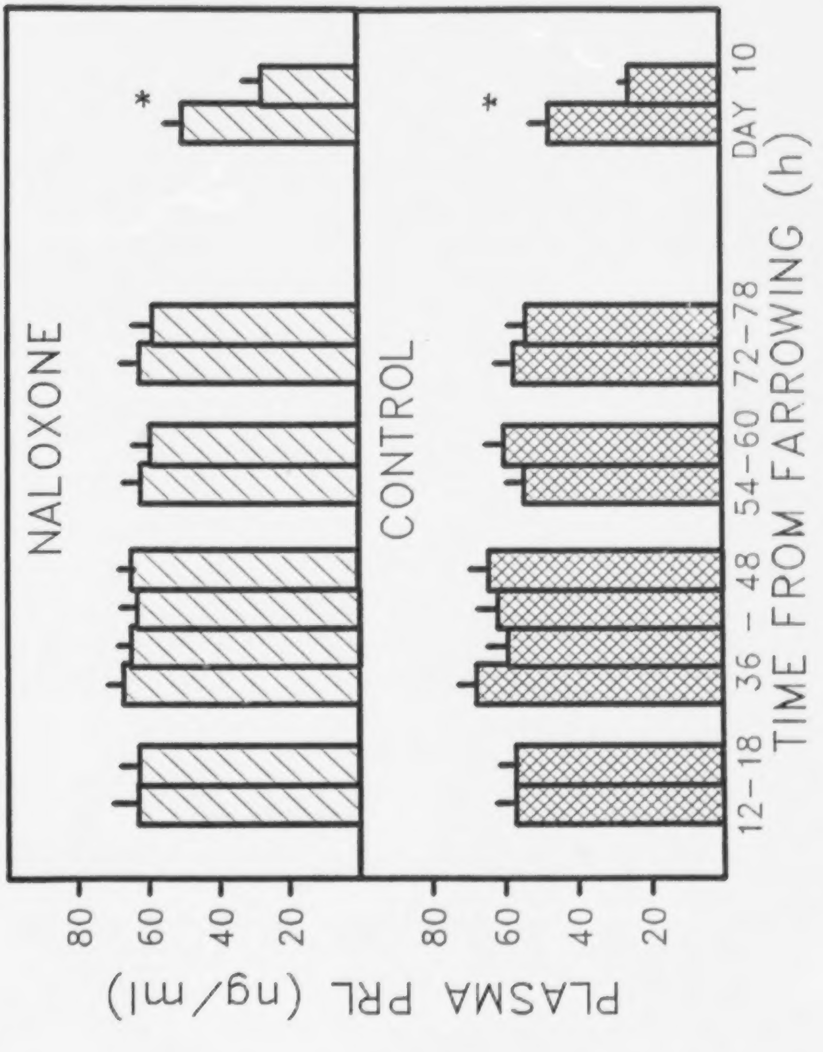

NALOXONE SOW A
NALOXONE SOW B
CONTROL SOW C
CONTROL SOW D
150
100
50
PLASMA PRL (ng/ml)
12-18
36 - 48
54-60
72-80
DAY10
TIME FROM FARROWING (h)

**Fig 2.7** Mean (+SEM) plasma prolactin (PRL) in Naloxone treated (n=6) and Control sows (n=5) in Experiment 1B. Each bar indicates the mean for 3h-periods of sampling. During the first 78h post-partum there was no effect of naloxone on plasma PRL. *, significant ($p<0.05$) decrease in PRL after naloxone injection on day 10 of lactation.


NALOXONE
CONTROL
*
*
PLASMA PRL (ng/ml)
80
60
40
20
80
60
40
20
12–18
36 – 48
54–60
72–78
DAY 10
TIME FROM FARROWING (h)

## 2.6 BIBLIOGRAPHY

Armstrong, J.D., Kraeling, R.R., Britt, J.H. (1986). Effect of bolus administration of naloxone on secretion of LH and prolactin in lactating sows. J. Anim. Sci. (Suppl 1) Abstract 322.

Armstrong, J.D., Kraeling, R.R., Britt, J.H. (1988). Effects of naloxone or transient weaning on secretion of LH and prolactin in lactating sows. J. Reprod. Fertil., 83:301-308.

Barb, C.R., Kraeling, R.R., Rampacek, G.B, Whisnant, C.S. (1986). Opioid inhibition of luteinizing hormone secretion in the post-partum lactating sow. Biol. Reprod., 35:368-371.

Barb, C.R., Kraeling, R.R., Rampacek, G.B., Leshin, L.S. (1987). Opioid modulation of follicle stimulating hormone (FSH) and prolactin (PRL) secretion in post-partum sow. In: Advances in Experimental Medicine and Biology. Ed. by V.B. Mahesh, D.S. Dhindsa, E. Anderson, S.P. Karla. Plenum, New York, pp. 647-652.

Barb, C.R., Kraeling, R.R., Rampacek, G.B. (1991). Opioid modulation of gonadotropin and prolactin secretion in domestic farm animals. Dom. Anim. Endocrinol., 91:15-27.

Ben-Jonathan, N., Arbogast, L.A., James, F.H. (1989). Neuroendocrine regulation of prolactin release. Progress in Neurobiology, 33:399-447.

Bevers, M.M., Willemse, M.H., Kruip, Th.A.M., Van de Wiel, D.F.M. (1981). Prolactin levels and the LH response to synthetic LHRH in lactating sow. Anim. Reprod. Sci., 4:155-163.

Bhanot, R., Wilkinson, M. (1984). The inhibitory effects of opiates on gonadotropin secretion is dependent upon gonadal steroids. J. Endocrinol., 102:133-141.

Britt, J.H., Armstrong, J.D., Cox, N.M. Esbenshade K. (1985). Control of follicular development during and after lactation in the sow. J. Reprod. Fertil., Suppl., 33:37-54.

Cosgrove, J.R., Booth, P.J., Foxcroft, G.R. (1991). Opioidergic control of gonadotrophin secretion in the prepubertal gilt during restricted feeding and realimentation. J. Reprod. Fertil., 91:277-284.

Currie, W.D., Rawlings, N.C. (1987a). Naloxone enhances LH but not FSH during various phases of the estrous cycle in the ewe. Life. Sci., 41:1207-1214.

Currie, W.D., Rawlings, N.C. (1987b). Fluctuation in responsiveness of LH and lack of responsiveness of FSH to prolonged infusion of morphine and naloxone in the ewe. J. Reprod. Fertil., 86:359-366.

De Rensis F., Hunter, M.G., Foxcroft, G.R. (1993). Suckling-induced inhibition of LH secretion and follicular development in the early post-partum sow. Biol. Reprod., 48:964-969.

Dondi, D., Maggi, R., Panerai, A., Piva, F., Limonta, P., (1991). Hypothalamic opiatergic tone during pregnancy,parturition and lactation in the rat. Neuroendocrinol., 53:460-466.

Ebling, F.J.P., Lincoln, G.A. (1985). Endogenous opioids and the control of seasonal LH secretion in Soay rams. J. Endocrinol., 107:341-353.

Ebling, F.J.P., Schwartz, M.L., Foster, D.L. (1989). Endogenous opioid regulation of pulsatile luteinizing hormone secretion during sexual maturation in the female sheep. Endocrinology, 125:369-383.

Grant, S.A., Hunter, M.G., Foxcroft, G.R. (1989). Morphological and biochemical characteristics during ovarian follicular development in the pig. J. Reprod. Fertil., 86:171-183.

Gregg, D.W., Moss, G.E., Hudgens, R.E., Malven, P.V. (1986). Endogenous opioid modulation of luteinizing hormone and prolactin in post-partum ewes and cows. J. Anim. Sci. 63:838-847.

Haynes, N.B., Lamming, G.E., Yang, K.P., Brooks, A.N., Finnie, A.D. (1989). Endogenous opioid peptides and farm animal reproduction. In: Oxford Reviews of Reproductive Biology. Ed., S.R. Milligan, 11:111-145.

Ieiri, T., Chen, H.T., Meites, J. (1980). Naloxone stimulation of luteinizing hormone release in prepubertal females rats, role of serotonergic system. Life. Sci., 26:1269-1274.

Knight, P.G., Howles, C.M., Cunningham, F.J. (1986). Evidence that opioid peptides and dopamine participate in the suckling-induced release of prolactin in the ewe. Neuroendocrinol., 44:29-35.

Kunavongkrit, A., Einarsson, S., Settergren, I. (1984). Follicular development in primiparous lactating sows. Anim. Reprod. Sci., 5:47-56.

Mattioli, M., Conte, F., Seren, E., Galeati, G. (1986). Effect of naloxone on plasma concentrations of prolactin and LH in lactating sows. J. Reprod. Fertil., 76:167-173.

Myers, T.R., Myers, D.A., Gregg, D.W., Moss, G.E. (1989). Endogenous opioid release of luteinizing hormone during suckling in post-partum anestrous beef cows. Dom. Anim. Endocrinol., 6:183-190.

Owens, D.P., Cicero, T.J., (1981). Development of acute tolerance to the effects of naloxone on the hypothalamic-pituitary-luteinizing hormone axis in the male rat. J. Pharmacol. Exp. Ther., 216:135-141.

Palmer, V.M., Teague, H.S., Venzke, W.G. (1965). Macroscopic observations on the reproductive tract of the sow during lactation and early post weaning. J. Anim. Sci., 24:541-545.

Pang, C.N., Zimmerman, E., Sawyer, C.H. (1977). Morphine inhibition of the preovulatory surge of plasma luteinizing hormone and follicle stimulating hormone. Endocrinology., 101:1726-1732.

Peters, A.R., Vyvoda, S., Lamming, G.E. (1979). Comparison of plasma prolactin levels in the milked and suckling cow. J. Endocrinol., 83:27P-28P.

Rawlings, N.C., Churchill, I.J., Currie, W.D., Joseph, I.B. (1991). Maturational changes in opioidergic control of luteinizing hormone and follicle stimulating hormone in ram lambs. J. Reprod. Fertil., 93:1-7.

Rojanasthien, S.A.M., Lundeheim, N., Einarsson, S. (1987). Luteinizing hormone response to different doses of synthetic gonadotrophin releasing hormone during early and late lactation in primiparous sows. Anim. Reprod. Sci., 13:299-307.

SAS. (1985) Sas User's Guide: Statistics Cary, NC: Statistical Analysis System Institute, Inc., Cary..

Shaw, H.J., Foxcroft, G.R. (1985). Relationship between LH, FSH, and prolactin secretion and reproductive activity in the weaned sow. J. Reprod. Fertil., 75:17-28.

Sirinathsinghji, D.J., Martini, L. (1984). Effects of bromocriptine and naloxone on plasma level of prolactin, LH and FSH during suckling in the female rat: response to gonadotropin releasing hormone. J. Endocrinol., 100:175-182.

Stevenson, J.S., Cox, N.M., Britt, J.H. (1981). Role of the ovary in controlling luteinizing hormone, follicle stimulating hormone and prolactin secretion during and after lactation in pigs. Biol. Reprod., 24:341-353

Trudeau, V.L., Meijer, J.C., Van de Wiel, D.F.M., Bevers, M.M., Erkens, J.H.F. (1988a). Effects of morphine and naloxone on plasma levels of LH, FSH, prolactin and growth hormone in the immature male pig. J. Endocrinol., 119:501-508.

Trudeau, V.L., Pharazyn, A., Beltranena, E., Aherne, F.X. (1988b). Naloxone elevates plasma follicle stimulating hormone but not luteinizing hormone levels in the immature male pig. Can. J. Anim. Sci., 69:1095-1098.

Varley, M.A., Foxcroft, G.R. (1990). Endocrinology of the lactating and weaned sow. J. Reprod. Fertil., Suppl., 40:47-61.

Whisnant, C.S, Kiser, R.E., Thompson, F.N., Barb, C.R. (1986a). Influence of calf removal on the serum luteinizing hormone response to naloxone in the postpartum beef cow. J. Anim. Sci., Suppl., 63: Abstract 561.

Whisnant, C.S., Kiser, R.E., Thompson, F.N., Barb, C.R. (1986b). Naloxone inhibition of luteinizing hormone secretion during the post-partum period in suckled beef cows. J. Anim. Sci., 63:1445-1448.

Wu, T., McArthur, N.H., Harms, P.G. (1992). A possible dual mechanism involved in the inhibitory influence of suckling on luteinizing hormone release in the ovariectomized rat. Biol. Reprod., 46:653-657.

# CHAPTER 3

## EXPERIMENT 2: OPIOID ANTAGONISM (NALOXONE) FAILS TO REVERSE THE SUCKLING-INDUCED REGULATION OF LH AND PROLACTIN SECRETION IN EARLY LACTATION IN THE SOW.

## 3.1 INTRODUCTION

The post-partum period in the sow is generally characterized by a suppression of LH secretion and a lack of follicular development (for review see Britt et al., 1985; Varley et al., 1990; Foxcroft, 1992). There is a substantial body of evidence supporting a role for endogenous opioids in the suppression of GnRH and LH secretion during lactation in pigs, sheep and cattle (for review see Haynes et al., 1989; Barb et al., 1991; De Rensis et al., 1993c; Britt, 1993). During lactation, the blockade of opiate receptors with naloxone also results in a decrease of PRL secretion in the rat (Bevers et al., 1981), sheep (Gregg et al.,1986) and sow (Mattioli et al., 1986; Barb et al., 1986; Armstrong et al., 1988b). In a previous study (Experiment 1, this thesis) we observed that chronic naloxone treatment was not able to prevent the suckling-induced inhibition of LH secretion in the early post-partum period but was effective in increasing LH at day 10 of lactation. These data provided evidence that opioidergic inhibition of LH secretion may not occur during the early post-partum period in the sow. However, the treatment regimen used also raised the possibility that a desensitization or down regulation of opioid receptors due to repeated naloxone treatment may have occurred.

Therefore, the aim of the present experiment was to use single injections of the opioid agonist naloxone at different times after farrowing to further define when the opioids become active in the control of LH secretion during lactation. Furthermore, by using chronic naloxone treatment from day 10 to day 11 of lactation (mid lactation), we investigated the likelihood that down regulation of naloxone receptors does occur after chronic naloxone treatment.

## 3.2 MATERIALS AND METHODS

### 3.2.1 Animals and blood collection

A total of 20 primiparous crossbred sows (Camborough x Canabrid, Pig Improvement (Canada) Ltd.) from the University of Alberta herd were used. Estrus was synchronized in groups of sows to facilitate bach farrowing. One group of ten animals farrowed in May and a second group of ten animals in June. Sows were housed in conventional farrowing crates and water and feed were provided ad libitum throughout lactation. The average weight of the sows one day after farrowing was 198.0±8.3kg and the litter size was standardized immediately after farrowing to 10 piglets/sow. Sows were provided surgically with indwelling jugular catheters via the cephalic vein under general anaesthesia 12-18h after parturition, allowing stress-free withdrawal of blood samples (2.5 ml) at 15min intervals during periods of sampling.

The experimental design is shown in Fig 3.1. The experiment was divided into two parts. In part I, to confirm the ontogeny of opioid-dependent regulation of LH and PRL secretion in early lactation, all animals were sampled over six hour periods from 36 to

42h, 48 to 54h, 60 to 66h and from 72-78h post-partum.

To facilitate the logistics of this experiment, sows were grouped as having completed farrowing within any particular 6h period. The end of each 6h period was then taken as time zero (0h post-partum). Groups of five sows were then allocated to receive 2mg/kg naloxone hydrochloride (Sigma-Tau) in saline i.v. for the first time three hours after the beginning of each period of sampling. As shown in Fig. 3.1, sow first treated at 39, 51 and 63h post-partum received three, two or one further injections, respectively, during subsequent period of sampling. Sows of group I received a single naloxone injection only at 75h post-partum. Within the subgroups therefore, repeated injections regimens were used to further elucidate the ontogeny of opioidergic regulation of LH secretion. Responses to naloxone treatment in these sows were compared to responses to single 2mg/kg i.v. injections of naloxone on either day 10 (n=10) or 11 (n=10) of lactation (see below).

In part II of the experiment all sows were sampled for 9h on day 10 and 7h on day 11 of lactation. To evaluate the possibility that repeated treatment with naloxone might result in decreased sensitivity to the opioid antagonist, 10 sows (Naloxone sows), allocated in a stratified manner to account for part I treatments, were treated with 2 mg/kg of naloxone hydrochloride (Sigma) in saline three hours after the start of sampling on day 10, followed by 10 further injections of 1mg/kg of naloxone at 3h intervals on days 10 and 11 of lactation. Ten control sows received saline vehicle only during sampling on day 10 and the first 4h on day 11 and then received a single i.v. injection of 2mg/kg of naloxone.

### 3.2.2 Hormone assays

Plasma LH concentrations were determined in all samples (15 min intervals) using the double antibody RIA described by De Rensis et al. (1993b). Inter- and intra-assay c.v were 12.7% and 4.1%, respectively. The sensitivity of the assay, defined as 90% of total binding, was 0.12 ng/ml.

Plasma concentrations of PRL were measured in samples drawn at 30 min intervals by the method described by Shaw and Foxcroft (1985) with minor modifications. Intra- and inter-assay c.v. were 3.9 and 10.7%, respectively. The overall sensitivity of the assay defined as 85% of total binding was 1.9 ng/ml.

### 3.2.3 Statistical analysis

Consideration of the plasma LH profiles in this study indicated that the adoption of computerized pulse analysis programs was inappropriate (for details see Foxcroft et al., 1988). Therefore to provide minimum, mean and maximum characteristics of LH secretion, LH profiles were analyzed by the method of Shaw and Foxcroft (1985) using a sliding window technique.

In part I of the experiment, two 3h means (before- and after-naloxone injections) were calculated for each 6h period of sampling. In part II, hormone concentrations during the 9h frequent sampling period at day 10 were analyzed as the means of three 3h periods; finally, the 7h frequent sampling period on day 11 was analyzed as means for three periods of 1h, 3h and 3h, respectively.

Part 1

Analysis of the effect of single injections of naloxone on mean LH and PRL plasma concentrations during each period were undertaken using the PROC GLM procedure within the SAS statistical package. A model fitting post-partum period (36 to 42h, 48 to 54h, 60 to 66h and from 72-78h post-partum) as a main effect and the effect of naloxone as a repeated measure was used. Analysis of LH or PRL responses to the second, third and fourth naloxone injections were similarly undertaken, the main effect of post-partum period being absent in the analysis of the response to the fourth injection.

Part 2

Analysis of responses to naloxone on days 10 and 11 was undertaken using a model fitting group (Naloxone or Control) as a main effect for each sampling period (d10: 3h, 3h, 3h, d11: 1h, 3h, 3h). Comparisons of responses to single and multiple injections of Naloxone on days 10 and 11 within Naloxone group sows were obtained fitting sampling period as a repeated measure. Having established significantly elevated LH concentrations in Control sows during pre-naloxone periods when compared with Naloxone sows, further analysis of the main effect of group was undertaken fitting the first 3h sampling period on d10 as a covariate. A comparison between LH or PRL concentrations during the periods prior to first naloxone injection in early lactation and on day 10 was undertaken for all sows utilising a repeat measures approach.

## 3.3 RESULTS

Plasma LH

The individual patterns of LH secretion in two sows in each sub-group are shown in Fig. 3.2-3.5.

Part 1

Single injections of 2mg/kg of naloxone for the first time at 39, 51, 63 and 75h post-partum did not affect minimum and mean plasma LH concentrations. An effect ($p<0.05$) of naloxone was observed 72-78h post partum in group IV sows treated three times previously with the antagonist, but not in the groups II and III treated once or twice previously, respectively (Fig. 3.6 and 3.7). Single and multiple naloxone injections on days 10 and 11 increased ($P<0.05$) mean LH concentrations in Control and Naloxone group sows, confirming previous observations of an EOP inhibition of LH secretion, vulnerable to antagonism by this dose of naloxone.

Part 2

There was no difference between LH concentrations prior to naloxone injections in early and mid (day 10) lactation. The LH responses to single and multiple naloxone injections did not differ between experimental groups (Naloxone or Control) for any periods except for the first and third period on d10 (Fig. 3.8). Further analysis of these differences, utilising the first 3h period on d10 as a covariate, established elevated LH concentrations in Naloxone sows in all except the final period on day 11, i.e. following administration of 2mg/kg naloxone to both groups. Repeated naloxone injections at 3h intervals between day 10 and 11 of lactation to Naloxone sows consistently increased mean LH concentrations ($p<0.05$).

Furthermore, the response to the 2mg/kg naloxone injections on day 11 in the

Naloxone group was not significantly different to the first injection of 2mg/kg naloxone on day 10 in the same sows, nor to the 2mg/kg naloxone injection on day 11 in the Control sows. Therefore, repetitive naloxone injections did not significantly suppress or apparently dampen the response to opioid antagonism.

Plasma PRL

The individual patterns of PRL secretion in two sows in each group are shown in Fig. 3.9-3.12 and mean PRL plasma concentrations during the first 78h post-partum in Fig. 3.13 and 3.14 and at days 10 and 11 of lactation in Fig. 3.15.

Part 1

Single injections of 2mg/kg of naloxone for the first time at 39, 51, 63 and 75h post-partum did not affect mean plasma PRL concentrations (Fig. 3.13 and 3.14). Responses to single and multiple naloxone injections on days 10 and 11 decreased ($p<0.05$) mean PRL concentrations in both Control and Naloxone group sows.

Part 2

There was no difference between PRL concentrations prior to naloxone injections in early and mid (day 10) lactation. Multiple naloxone injections between day 10 and 11 of lactation consistently suppressed mean plasma PRL ($p<0.05$) (Fig. 3.15). Furthermore, the response to the 2mg/kg naloxone injections on day 11 in the naloxone group was not significantly different to the first injection of 2mg/kg naloxone on day 10 in the same sows, nor to the 2mg/kg naloxone injection on day 11 in the control sows. Therefore, similarly to LH, repetitive naloxone injections did not significantly suppress or apparently dampen the response to opioid antagonism.

## 3.4 DISCUSSION

A gradual suppression of LH secretion with time from farrowing has been reported previously (Dondi et al., 1991; Tokach et al., 1992; Sesti et al., 1993). In an earlier study we observed that inhibition of LH secretion only becomes established in suckled sows around 66-78h post-partum, while in sows weaned at birth, LH secretion is maintained beyond this time and is associated with immediate follicular development (De Rensis et al., 1993a). The existence of active LH secretion in the immediate post-partum period and a gradual suppression of basal LH concentrations as lactation progressed, was also confirmed in a previous study using naloxone (De Rensis et al., 1993b). In contrast, in the present study, mean plasma LH was not different between the immediate post-partum periods (36-78h) compared with day 10 and 11 of lactation. The bleeding schedule adopted did not require blood sampling before 36h after parturition and it appears that in these animals, LH secretion was already inhibited at this time. Similarly Smith et al. (1992) did not observe differences in mean LH concentrations between day 1 and day 14 post-partum.

During the early post-partum period single naloxone injections of 2mg/kg only increased plasma LH in Group IV sows treated with a fourth injection 75h post-partum but not during the three previous treatments with the antagonist in this group. In contrast, LH secretion was increased in all sows after naloxone treatment at day 10 and 11 of lactation. These data support our previous suggestion (De Rensis et al., 1993b) that endogenous opioids do not mediate the initial inhibitory effects of suckling on LH secretion in the early post-partum period. However, an inhibitory opioidergic regulation of LH secretion becomes active after this time and is consistently demonstrable in established lactation. Evidence for differential opioidergic control of LH secretion in the early and later post-partum periods has also been recently reported from studies in lactating rats (Wu et al., 1992). Therefore, we suggest that LH release in the early post-partum period may be regulated by an alternative, non-opioidergic, mechanism. It is also possible that LH release in the early post-partum period is subject to opioidergic inhibition that cannot be antagonized by the dosage of naloxone utilized in this experiment or that effects on LH could be mediated by opioid receptors not readily antagonized by naloxone. However, naloxone binds to mu, delta and kappa receptors in brain tissue (Chang, 1984) and it has been reported that opioids implicated in the suppression of LH are of mu type. It would still be of interest to further investigate the presence and type of opioid receptors involved in the control of GnRH secretion during early lactation.

Consistent with our own previous work (De Rensis et al., 1993b) and other studies (Armstrong et al., 1986; Barb et al., 1986, 1991; Mattioli et al., 1986; Whisnant et al., 1986; Gregg et al., 1986), three sows of the Control group and two sows of the Naloxone group did not respond to naloxone treatment at day 10 and 11 of lactation. The cause of this lack of responsiveness continues to be uncertain. In rats (Owens and Cicero, 1981) and in male sheep (Ebling and Lincoln, 1985) it has been reported that naloxone administration is associated with a decline in the LH response following subsequent injections of naloxone. Similarly, Knight et al. (1986) did not obtain a significant naloxone-induced increase of serum LH in suckled ewes by repetitive administration of naloxone in small doses (4.17 mg/ewe every 5min for a 75min period). Conversely, Myers et al. (1989) in beef cows and Rawlings et al. (1991) in rams, did not observe any inhibitory effect on LH secretion of repeated naloxone injections when treating the animals at 2h intervals.

In our experiment from day 10 through day 11 of lactation, naloxone was injected every three hours and an effect of naloxone on LH secretion existed throughout the period of treatment. Therefore our study indicates that the injection of naloxone in lactating sows at three hourly intervals does not induce a desensitization of opioid receptors. These data suggest that the lack of a response to naloxone treatment in the immediate post-partum period in our previous study (De Rensis et al., 1993b) was not a result of the multiple injection schedule adopted.

From the present study it is not possible to describe the level at which naloxone affects LH secretion. However in vivo and in vitro studies in a variety of species showed that naloxone did not alter the pituitary LH responses to pulses of GnRH (Grossman et al., 1989; Ferin et al., 1984; Ebling et al., 1987) and did not disrupt the secretory response of the pituitary to exogenous GnRH given in vivo (Cicero et al., 1977; Grossman et al., 1989; Ferin et al., 1984). Finally, Kalra et al. (1984) described that intracerebral injection of naloxone stimulates LH release in a similar manner to that seen after systemic naloxone injections. There are, however, recent data showing that LH secretion can be modulated by a direct effect of opioids at the pituitary level (Chao et al., 1986; Blank et al., 1986; Mattery and Moberg, 1985; Barb et al., 1990).

The significant decline in PRL secretion after naloxone treatment at day 10 and 11 of lactation in most sows was consistent with data from previous studies in lactating sows, which established an effect of naloxone injection on plasma PRL in sows treated at days 10 or 11 (De Rensis et al., 1993b), day 15 (Mattioli et al., 1986), day 21 (Armstrong et al., 1988b) and at day 22 (Barb et al., 1986) post-partum. However, naloxone treatment did not change plasma PRL during the first 78h after farrowing, confirming our previous studies (De Rensis et al., 1993c) and studies in ewes (Gregg et al., 1986) in which a decrease in serum concentrations of PRL after naloxone treatment (1.0mg/kg) was observed on day 14 to 26 but not on day 10 post-partum. Therefore, these data seem to suggest that the regulation of PRL secretion during the early post-partum period is also not sensitive to naloxone treatment.

## 3.5 CONCLUSION

The ability of both single and chronic naloxone treatment to counteract the suppression of LH at days 10 and 11 of lactation in the sow, but the lack of an effect of naloxone in most of the treated animals during the first 78h post-partum, indicates that inhibitory opioidergic systems may require a specific time to become operative. Similarly, the observation that PRL secretion during the early post-partum period was not modified by naloxone but that a significant decrease was evident at day 10 and 11 of lactation, indicates that there are also temporal differences in the effect of endogenous opioids on PRL secretion during lactation.

**Fig. 3.1** Schematic diagram of experimental design. Arrows indicate time of naloxone injections. Part I comprised 4 sub-groups (I-IV) of five sows each and part II comprised the same sows split between two groups (Nalox and Control) of ten sows.

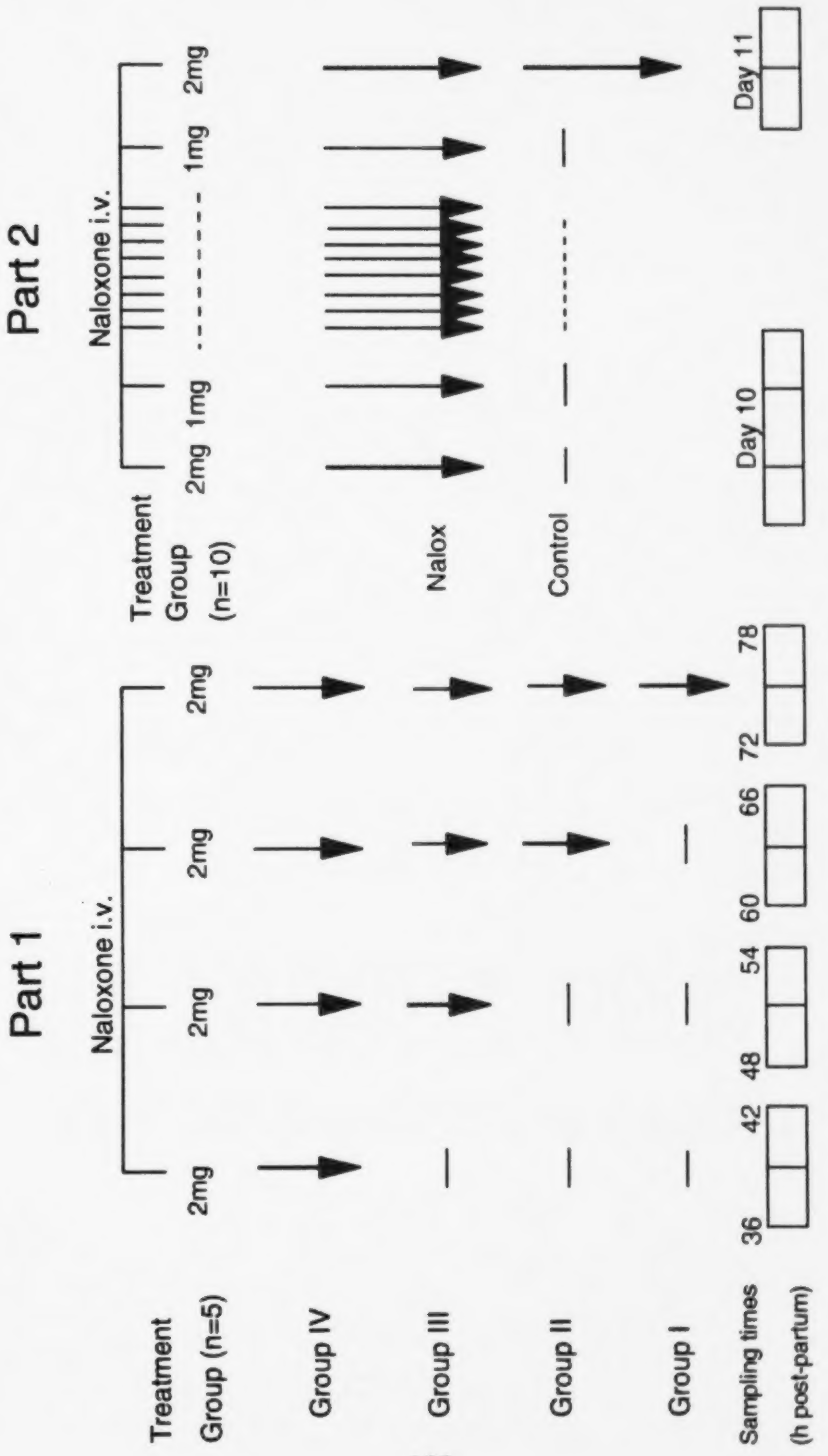
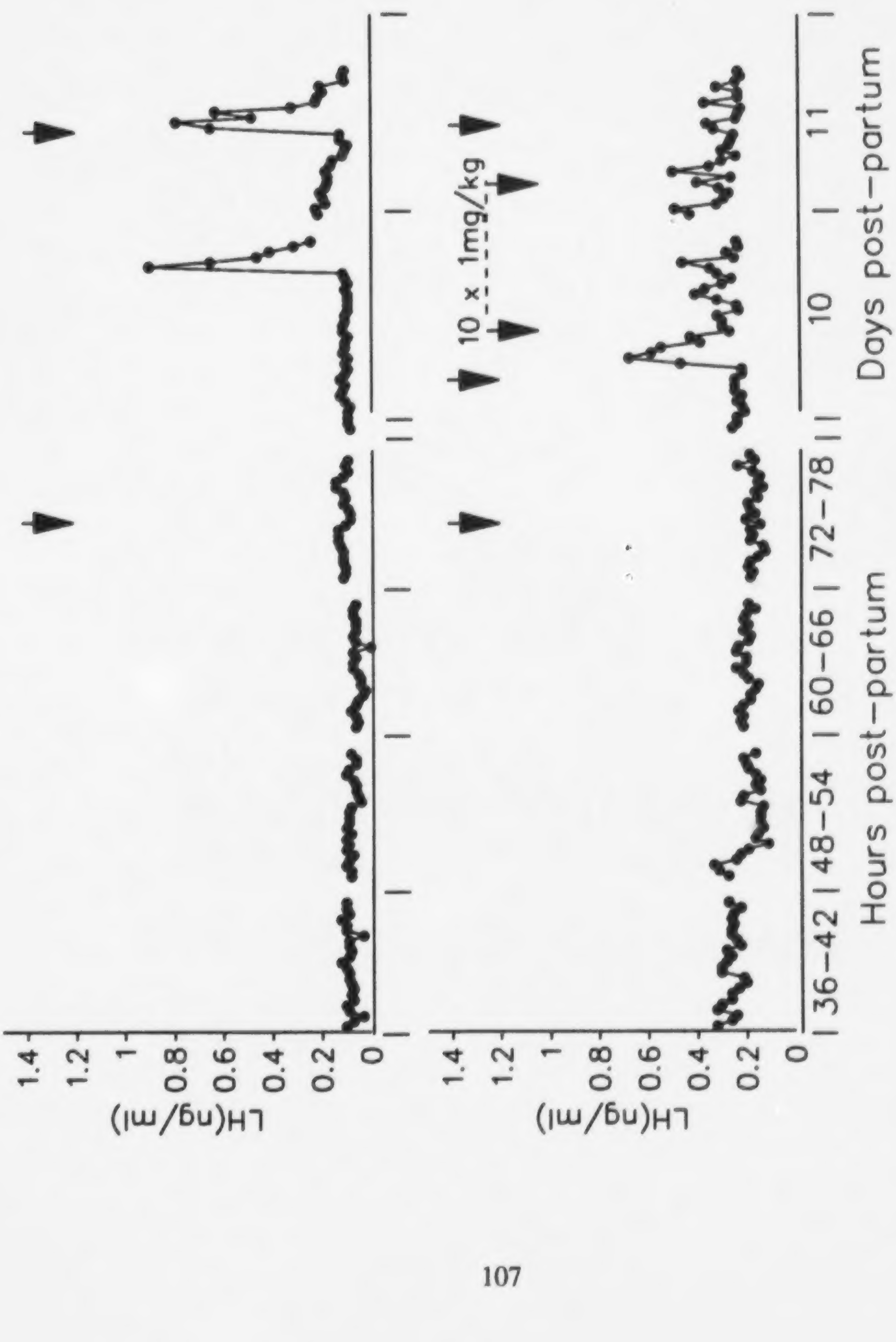

Part 1
Naloxone i.v.
Treatment
Group (n=5)
2mg
2mg
2mg
2mg
Group IV
Group III
Group II
Group I
Sampling times
(h post-partum)
36
42
48
54
60
66
72
78
Part 2
Naloxone i.v.
Treatment
Group
(n=10)
2mg
1mg
1mg
2mg
Nalox
Control
Day 10
Day 11

**Fig. 3.2** Individual plasma LH profiles in two sows first treated with single naloxone injections 75h post-partum (group I). Arrows indicate naloxone injections.



LH(ng/ml)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
LH(ng/ml)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
10 x 1mg/kg
36–42
48–54
60–66
72–78
Hours post-partum
10
11
Days post-partum

**Fig. 3.3** Individual plasma LH profiles in two sows first treated with single naloxone injections 63h post-partum (group II). Arrows indicate naloxone injections.

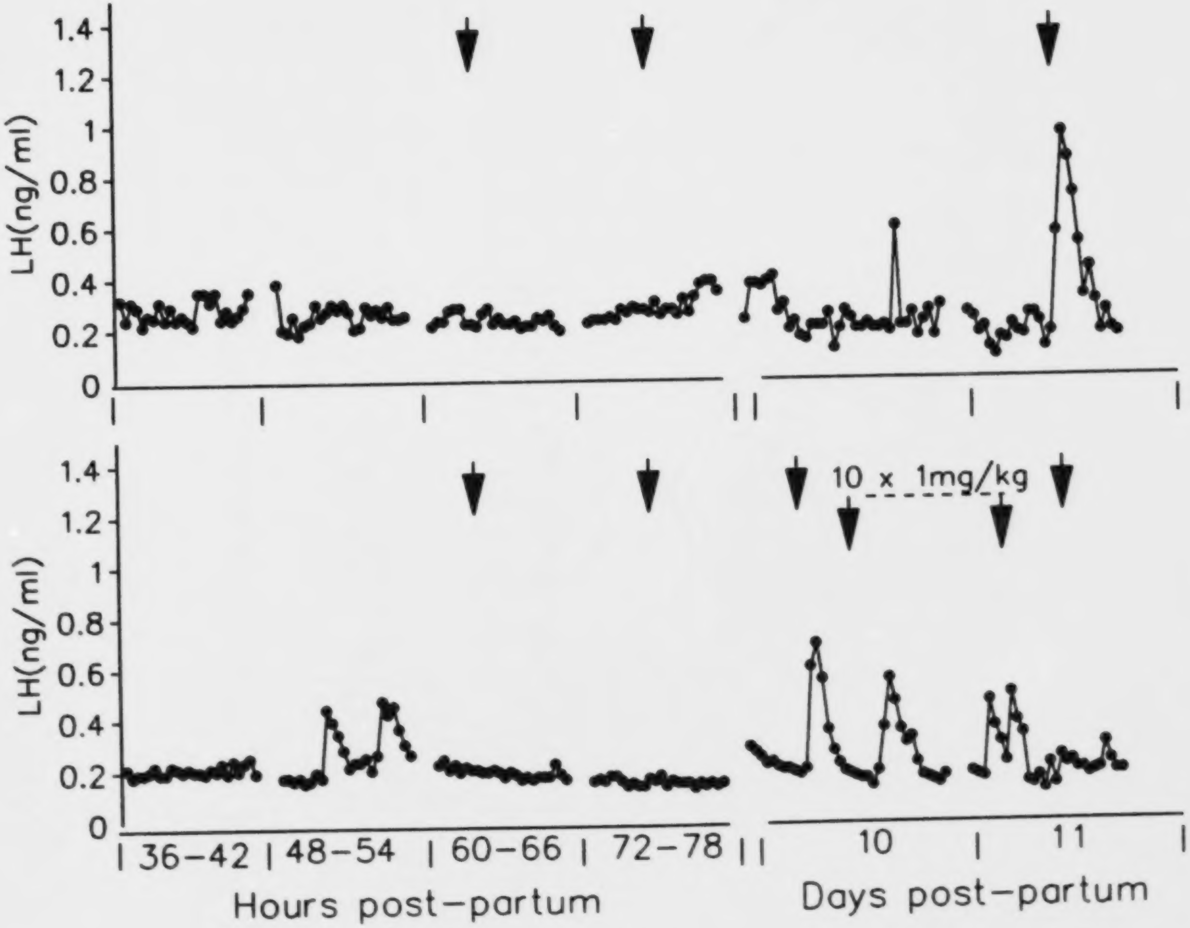

LH(ng/ml)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
LH(ng/ml)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
10 x 1mg/kg
36–42
48–54
60–66
72–78
10
11
Hours post–partum
Days post–partum

**Fig. 3.4** Individual plasma LH profiles in two sows first treated with single naloxone injections 51h post-partum (group III). Arrows indicate naloxone injections.

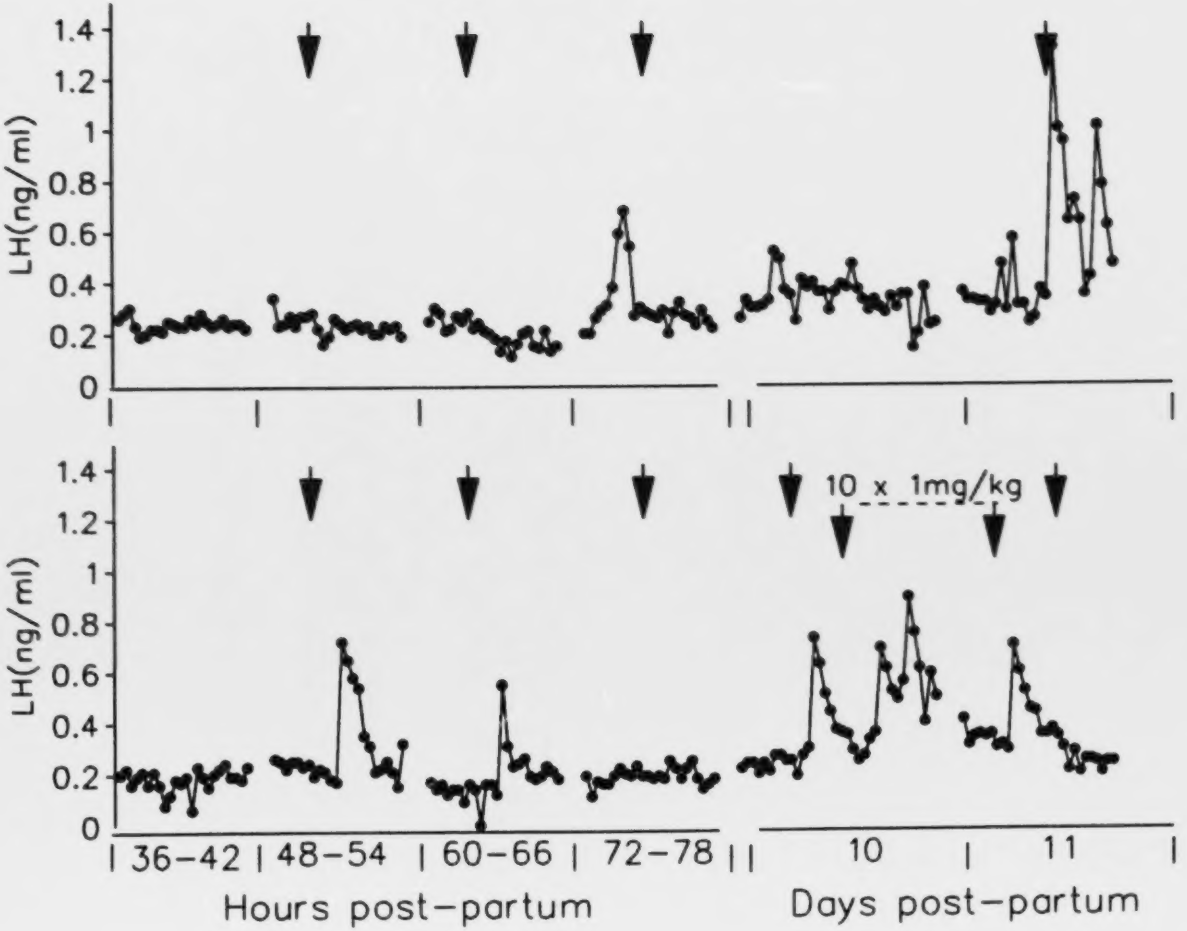

1.4
1.2
1
0.8
0.6
0.4
0.2
0
LH(ng/ml)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
LH(ng/ml)
10 x 1mg/kg
36-42
48-54
60-66
72-78
10
11
Hours post-partum
Days post-partum

**Fig. 3.5** Individual plasma LH profiles in two sows first treated with single naloxone injections 39h post-partum (group IV). Arrows indicate naloxone injections.

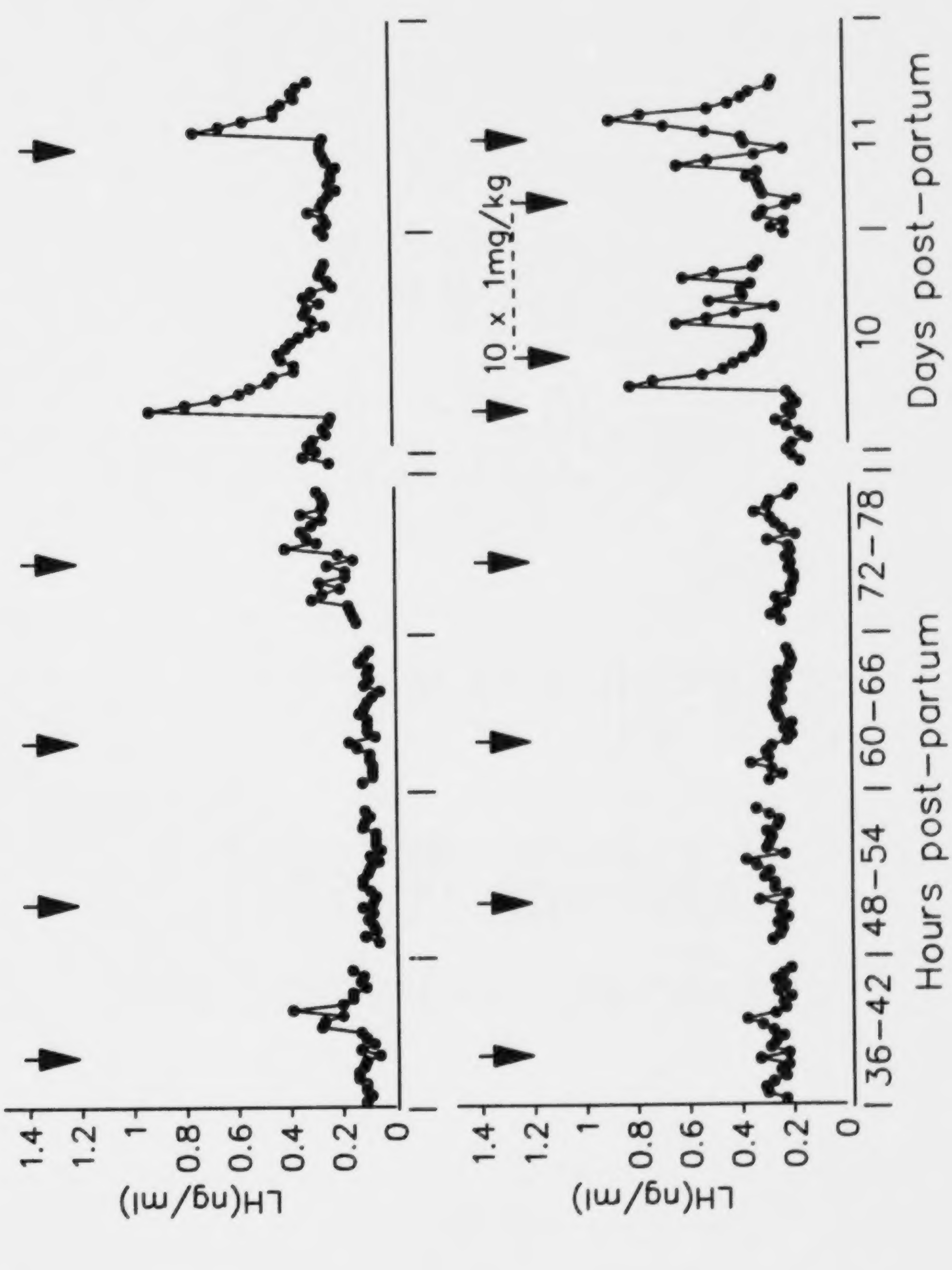
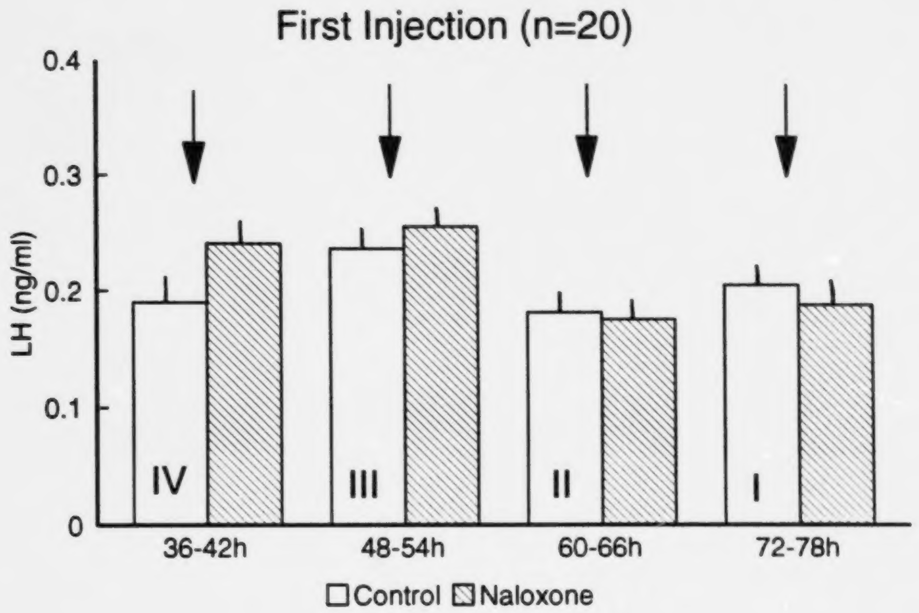

LH(ng/ml)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
LH(ng/ml)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
36–42
48–54
60–66
72–78
Hours post-partum
10 x 1mg/kg
10
11
Days post-partum

**Fig. 3.6** Mean (+SEM) plasma LH concentrations during control pre-treatment periods, and in response to the first (top panel) or second (lower panel) naloxone (2mg/kg) injections given during different post-partum periods. Arrows indicate naloxone injections in different treatment groups (I-IV, see Fig. 2.1.). No significant (P>0.05) responses to treatment were established.


First Injection (n=20)

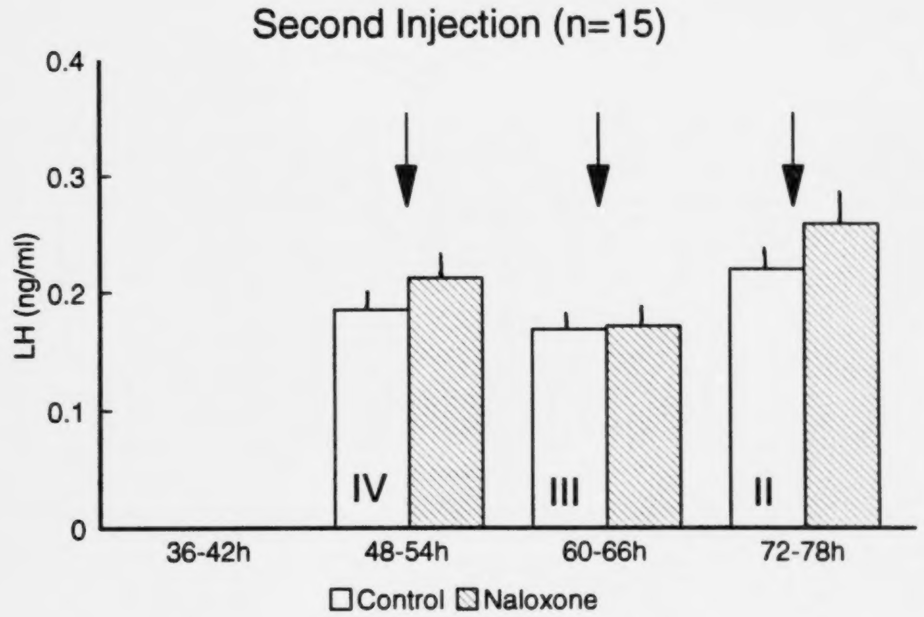
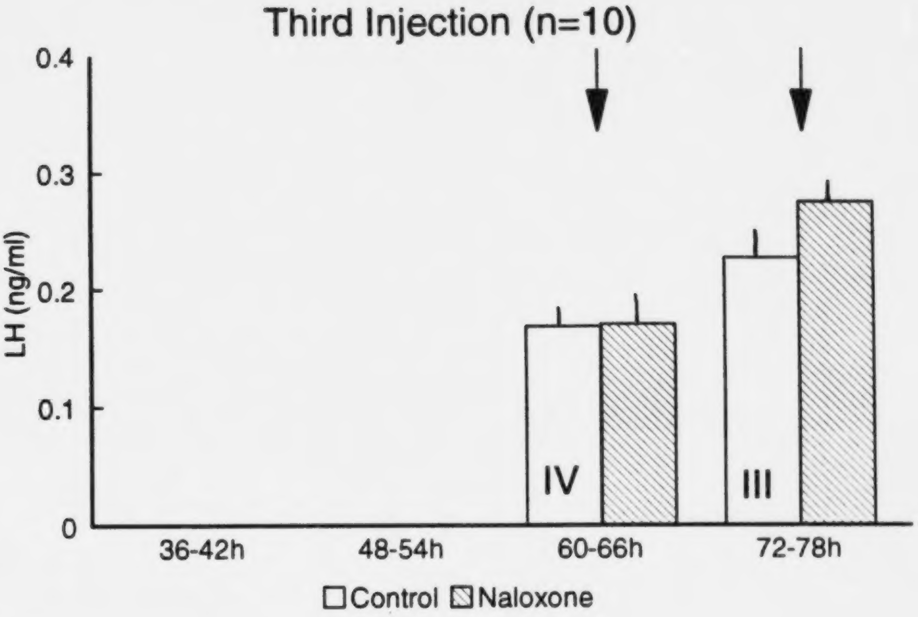

Second Injection (n=15)

**Fig. 3.7** Mean plasma LH concentrations during control pre-treatment periods, and in response to the third or fourth naloxone (2mg/kg) injections given during different post-partum periods. Arrows indicate naloxone injections. * Significant response to naloxone ($P<0.05$).


Third Injection (n=10)

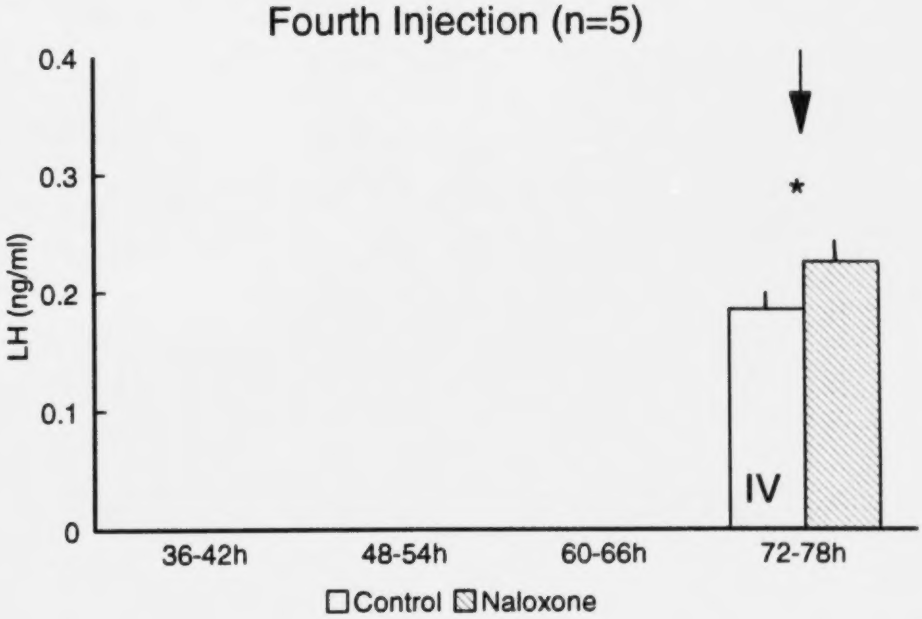
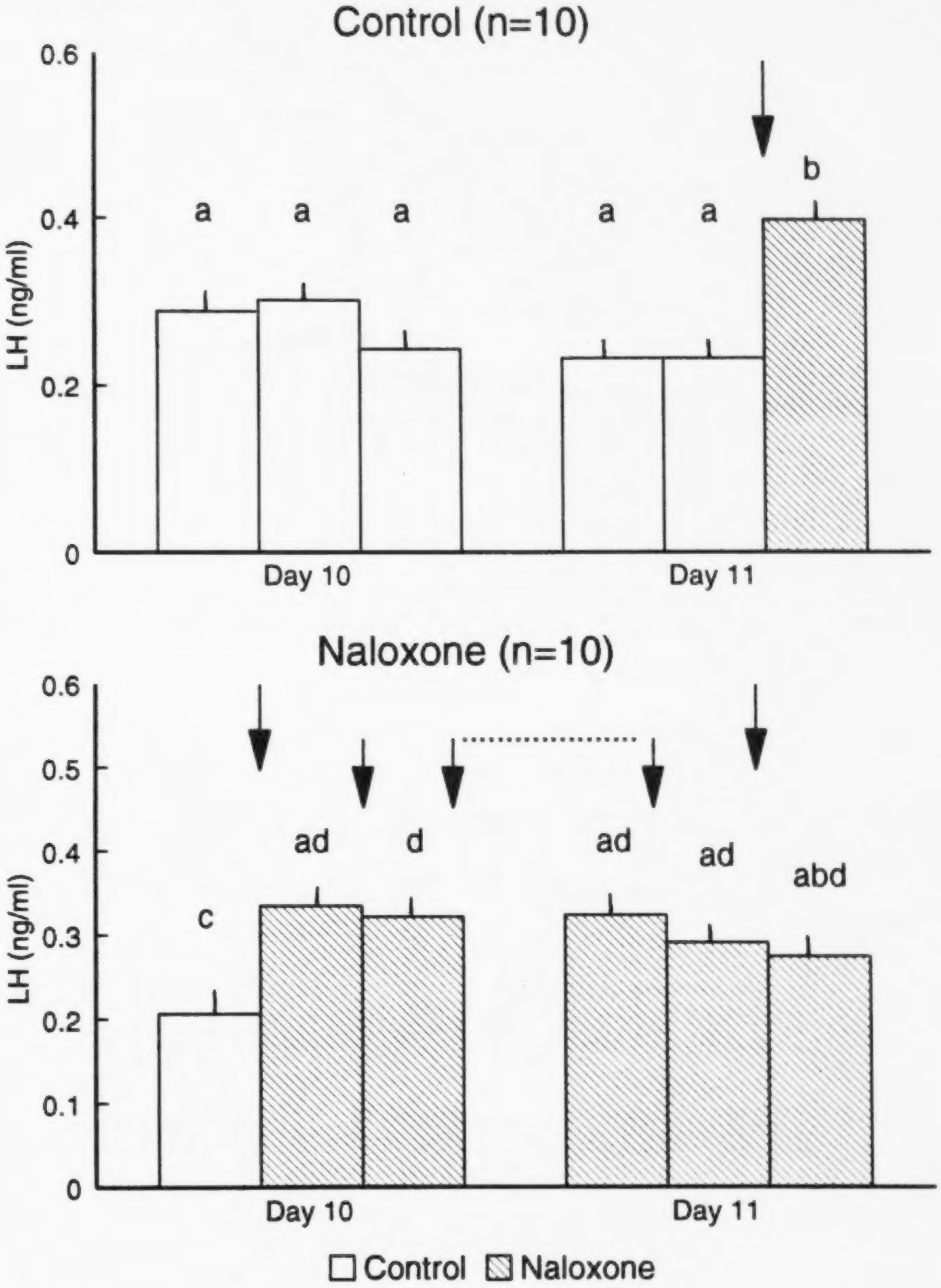

Fourth Injection (n=5)

**Fig. 3.8** Mean (+SEM) plasma LH concentrations in Control and Naloxone groups for three bleeding periods on days 10 (3h, 3h, and 3h) and 11 (1h, 3h and 3h) of lactation. Arrows indicate naloxone injections. Different superscripts denote differences ($p<0.05$) between period means within groups and between groups within time of sampling.



Control (n=10)

Naloxone (n=10)

**Fig. 3.9** Individual plasma PRL profiles in two sows first treated with single naloxone injections 75h post-partum (group I). Arrows indicate naloxone injections.

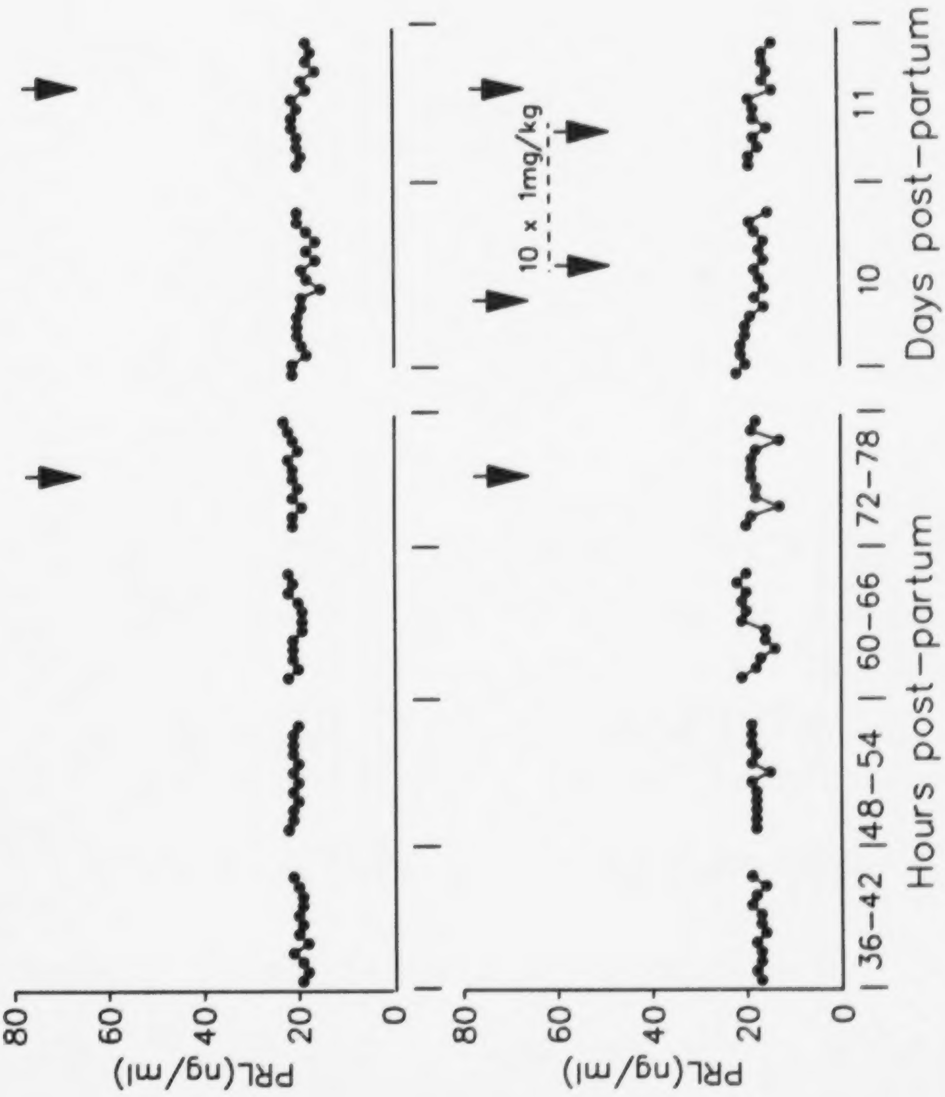

PRL(ng/ml)
80
60
40
20
0
PRL(ng/ml)
80
60
40
20
0
36–42
48–54
60–66
72–78
Hours post–partum
10 x 1mg/kg
10
11
Days post–partum

**Fig. 3.10** Individual plasma PRL profiles in two sows first treated with single naloxone injections 63h post-partum (group II). Arrows indicate naloxone injections.

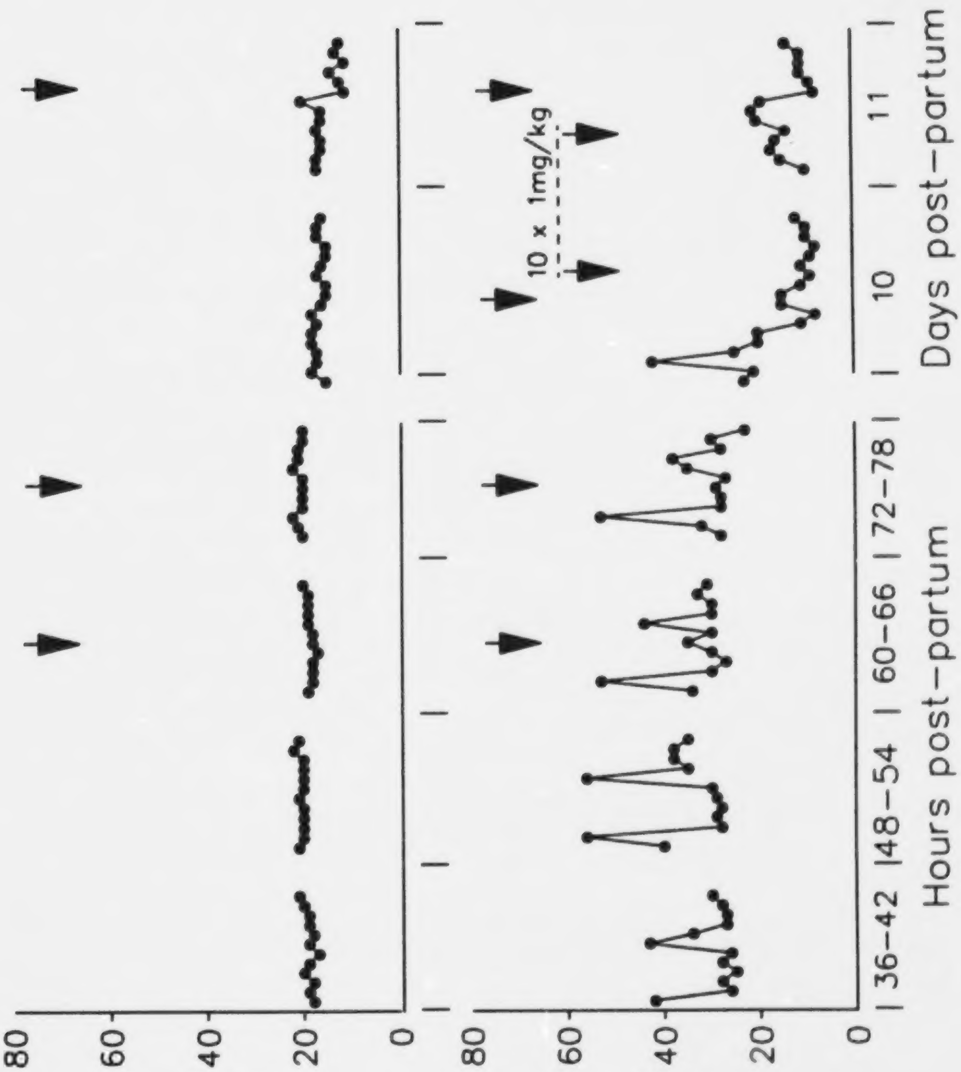

PRL(ng/ml)
0
20
40
60
80
PRL(ng/ml)
0
20
40
60
80
36-42
48-54
60-66
72-78
Hours post-partum
10 x 1mg/kg
10
11
Days post-partum

**Fig. 3.11** Individual plasma PRL profiles in two sows first treated single naloxone injections 51h post-partum (group III). Arrows indicate naloxone injections.

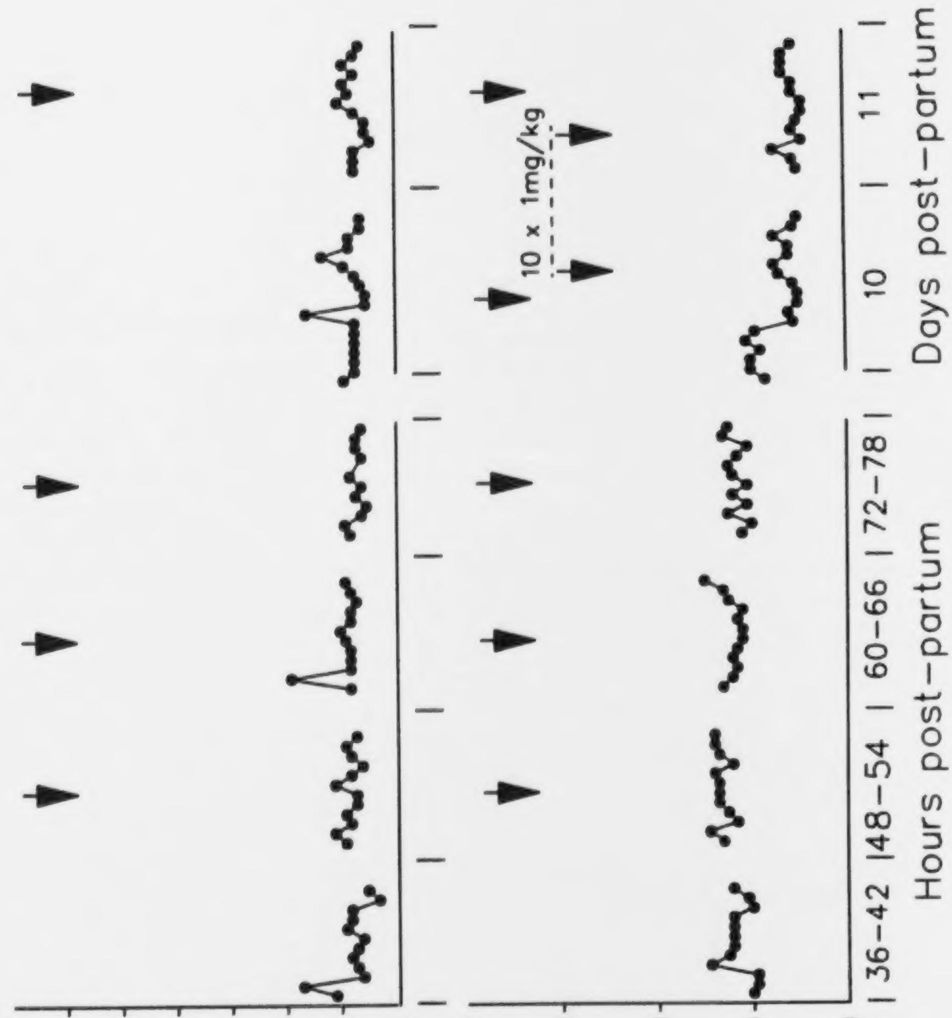

PRL(ng/ml)
80
70
60
50
40
30
20
10
PRL(ng/ml)
80
60
40
20
0
10 x 1mg/kg
36-42
48-54
60-66
72-78
Hours post-partum
10
11
Days post-partum

**Fig. 3.12** Individual plasma PRL profiles in two sows first treated with naloxone injections 39h post-partum (group IV). Arrows indicate naloxone injections.

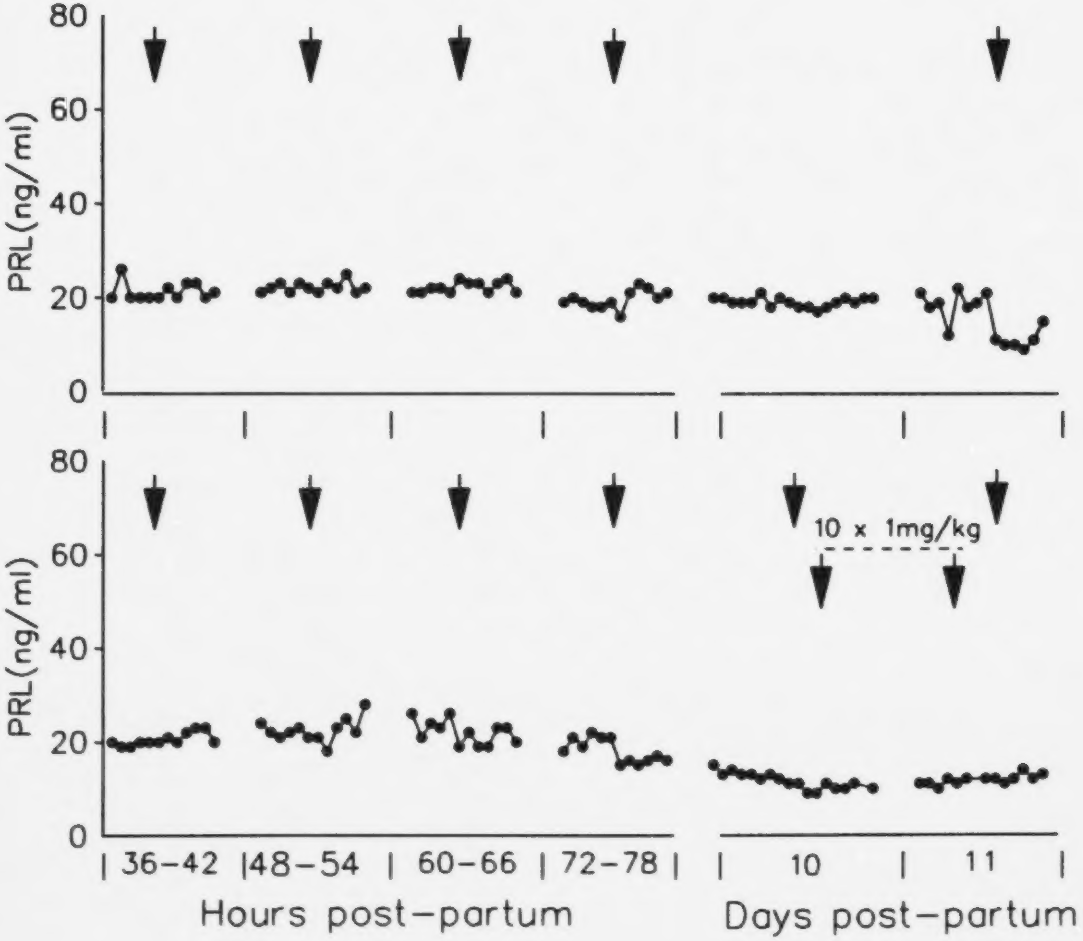
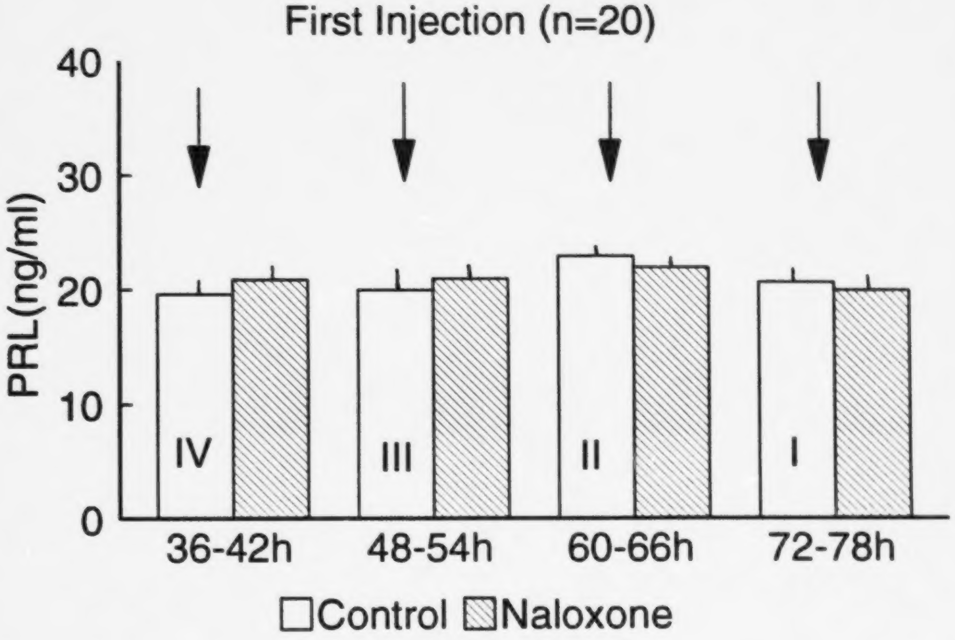

80
60
40
20
0
PRL(ng/ml)
80
60
40
20
0
PRL(ng/ml)
10 x 1mg/kg
36–42
48–54
60–66
72–78
10
11
Hours post–partum
Days post–partum

**Fig. 3.13** Mean (+SEM) plasma PRL concentrations during control pre-treatment periods and in response to the first or second naloxone (2mg/kg) injections given during different post-partum periods in different groups of sows (I-IV, see fig. 2.1.). Arrows indicate naloxone injections. There were no significant ($P>0.05$) responses to naloxone.


First Injection (n=20)
PRL(ng/ml)
40
30
20
10
0
IV
III
II
I
36-42h
48-54h
60-66h
72-78h
☐Control ▨Naloxone

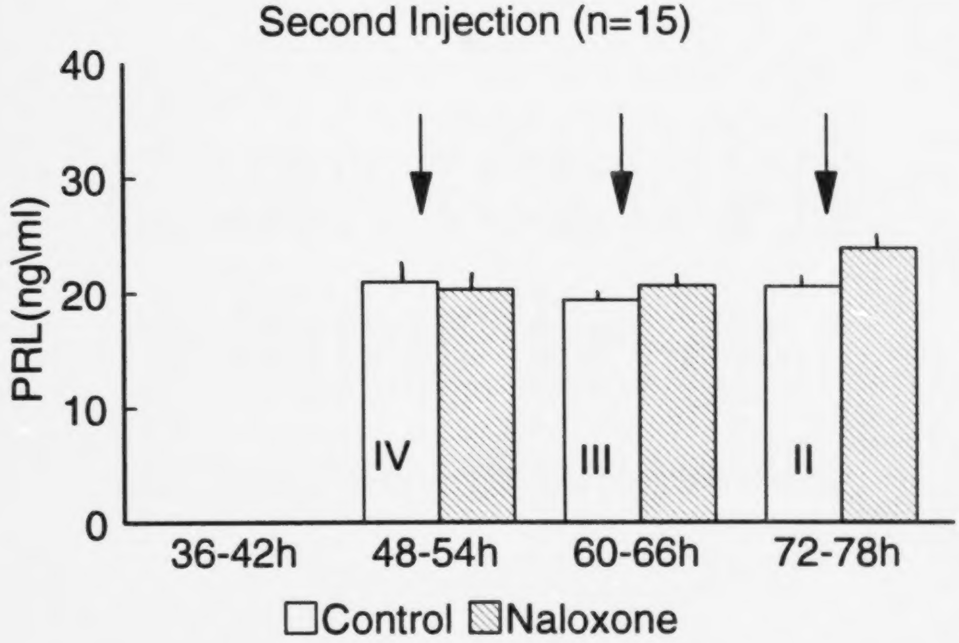
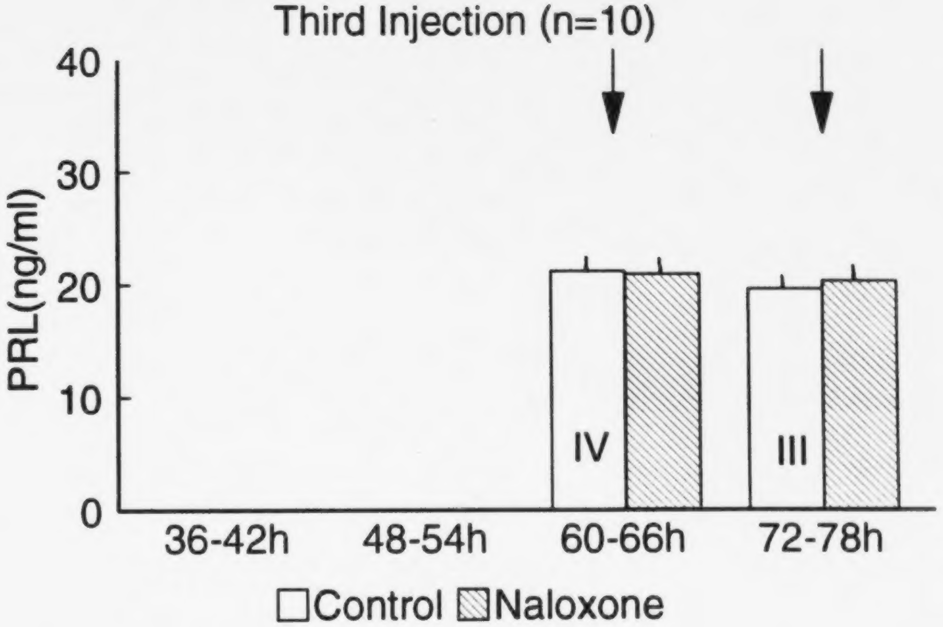
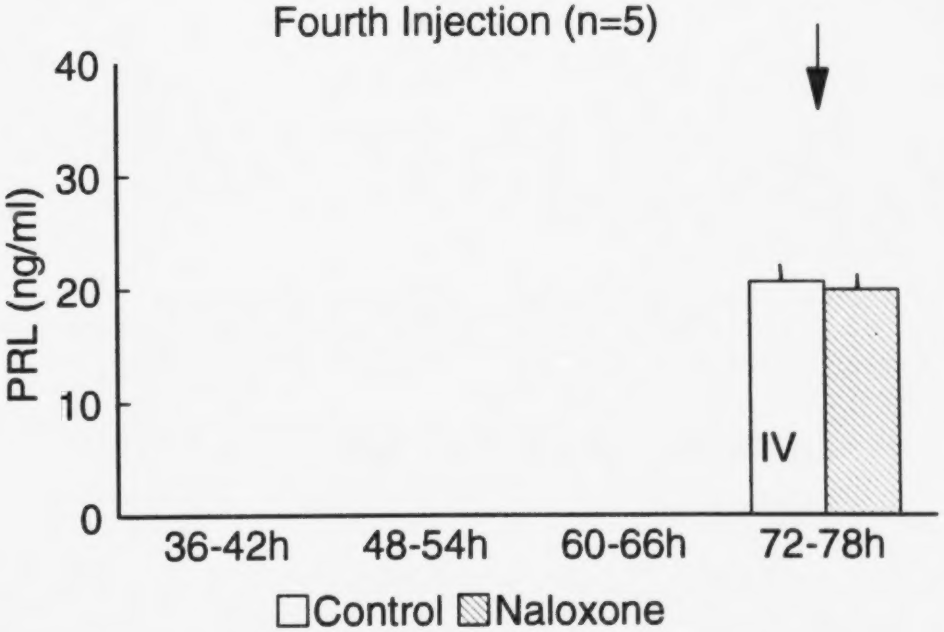

Second Injection (n=15)
PRL(ng\ml)
40
30
20
10
0
IV
III
II
36-42h
48-54h
60-66h
72-78h
☐Control ▨Naloxone

**Fig. 3.14** Mean (+SEM) plasma PRL concentrations during control pre-treatment periods and in response to the third or fourth naloxone (2mg/kg) injections given during different post-partum periods in different groups of sows (I-IV, see fig 2.1.). Arrows indicate naloxone injections. There were no significant ($P<0.05$) responses to naloxone treatment.

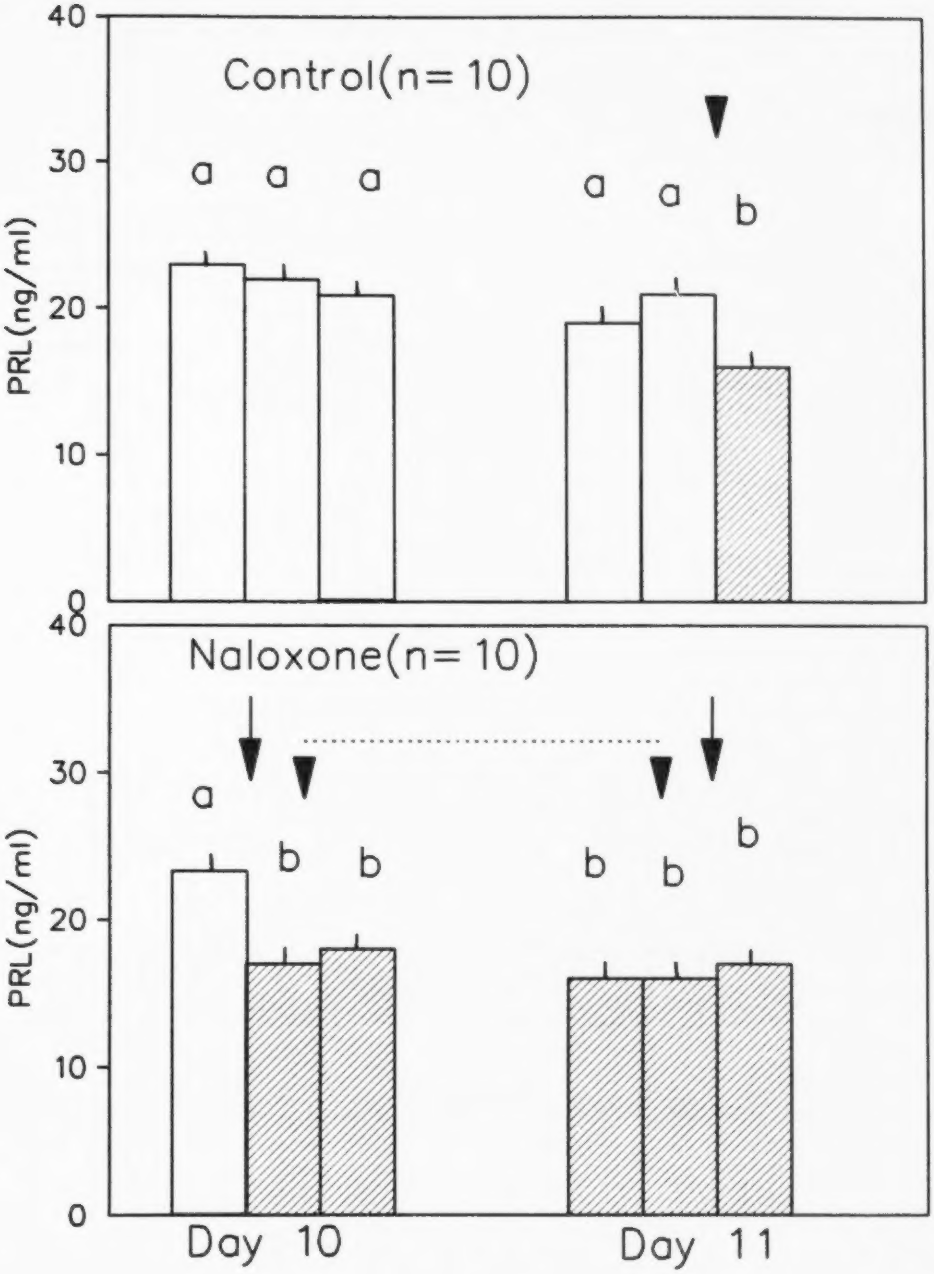


Third Injection (n=10)
PRL(ng/ml)
40
30
20
10
0
IV
III
36-42h
48-54h
60-66h
72-78h
☐Control ▨Naloxone



Fourth Injection (n=5)
PRL (ng/ml)
40
30
20
10
0
IV
36-42h
48-54h
60-66h
72-78h
☐Control ▨Naloxone

**Fig. 3.15** Mean (+SEM) plasma PRL concentrations in Control and Naloxone groups for three bleeding periods on days 10 (3h,3h, and 3h) and 11 (1h, 3h and 3h). Arrows indicate naloxone injections. Different superscripts denote differences ($p<0.05$) between period means and between groups within sampling periods.


Control(n= 10)
a
a
a
a
a
b
PRL(ng/ml)
0
10
20
30
40
Naloxone(n= 10)
a
b
b
b
b
b
PRL(ng/ml)
0
10
20
30
40
Day 10
Day 11

## 3.6 BIBLIOGRAPHY

Armstrong, J.D., Kraeling, R.R., Britt, J.H. (1986). Effect of bolus administration of naloxone on secretion of LH and prolactin in lactating sows. J. Anim. Sci., (Suppl. 1) Abstract 322.

Armstrong, J.D., Kraeling, R.R., Britt, J.H. (1988a). Morphine suppresses luteinizing hormone concentrations in transiently weaned sows and delays onset of estrus after weaning. J. Anim. Sci., 66:2216-2222.

Armstrong, J.D., Kraeling, R.R., Britt, J.H. (1988b) Effects of naloxone or transient weaning on secretion of LH and prolactin in lactating sows. J. Reprod. Fertil., 83:301-308.

Barb, C.R., Kraeling, R.R., Rampacek, G.B., Whisnant, C.S. (1986). Opioid inhibition of luteinizing hormone secretion in the post-partum lactating sow. Biol. Reprod., 35:368-371.

Barb, C.R., Kraeling, R.R., Rampacek, G.B. (1991). Opioid modulation of gonadotropin and prolactin secretion in domestic farm animals. Dom. Anim. Endocrinol., 91:15-27.

Bevers, M.M., Willemse, M.H., Kruip, Th. A.M., Van de Wiel, D.F.M. (1981). Prolactin levels and the LH response to synthetic LHRH in lactating sow. Anim. Reprod. Sci., 4:155-163.

Blank, M.S., Fabbri, A., Catt, K.J., Dufau, M.L. (1986). Inhibition of luteinizing hormone release by morphine and endogenous opiates in cultured pituitary cells. Endocrinology., 118:2097-2101.

Britt, J.H., Armstrong, J.D., Cox, N.M., Esbenshade, K. (1985). Control of follicular development during and after lactation in the sow. J. Reprod. Fertil., Suppl. 33:37-54

Barb, C.R., Barret, J.B., Wright, J.T., Kraeling, R.R., Rampacek, G.B. (1990). Opioid modulation of LH secretion by pig pituitary cells in vitro. J. Reprod. Fertil., 90:213-218.

Britt, J.H., Armstrong, J.D., Moore, K.L., Sesti, L.A.C. (1993). Involvement of opioids in regulation of LH secretion during lactational- or nutritional-induced anestrus in pig and cattle. In: Opioids in farm animals, Ed. N.Parvizi, Landwirtschaftsverlag GmbH, 4400 Münster-Hiltrup, Germany. pp. 34-54.

Chang, K.J. (1984). Opioid receptors: Multiplicity of ligand-receptors interactions. I: The receptors. Eds. P.M. Conn. Academic Press, New York, 1:1-81.

Chao, C.C., Malven, P.V., Moss G.E. (1986). Direct opioid regulation of pituitary release of bovine luteinizing hormone. Life Sci., 39:527-534.

Cicero, T.J., Bordger, T.M., Willcox, C.E., Bell, R.D., Meyer, E.R. (1977). Morphine decreases luteinizing hormone by an action on the hypothalamic-pituitary axis. J. Pharmacol. exp. Therapeutics, 203:548-555.

Cosgrove, J.R., Booth, P.J. and Foxcroft, G.R. (1991). Opioidergic control of gonadotrophin secretion in the prepubertal gilt during restricted feeding and realimentation. J. Reprod. Fertil., 91:277-284.

De Rensis, F., Hunter, M.G., Foxcroft, G.R. (1993a). Suckling-induced inhibition of LH secretion and follicular development in the early post-partum sow. Biol. Reprod., 48:964-969.

De Rensis, F., Cosgrove, J.R. Foxcroft, G.R., (1993b). LH and Prolactin responses to naloxone vary with stage of lactation in the sow. Biol. Reprod., 48:970-976.

De Rensis, F., Foxcroft, G.R. (1993c) Endogenous opioid modulation of gonadotropin and prolactin secretion in the post-partum ewe. In: Opioids in farm animals, Ed. N. Parvizi, Landwirtschaftsverlag GmbH, 4400 Münster-Hiltrup, Germany. pp. 15-33.

Dondi, D., Maggi, R., Panerai, A., Piva, F., Limonta, P. (1991). Hypothalamic opiatergic tone during pregnancy, parturition and lactation in the rat. Neuroendocrinol., 53:460-466.

Ebling, F.J.P., Lincoln, G.A. (1985). Endogenous opioids and the control of seasonal LH secretion in Soay rams. J. Endocrinol. 107:341-353.

Ferin, M., Van Vugt, D., Wardlaw, S. (1984). The hypothalamic control of the menstrual cycle and the role of endogenous opioid peptides. Rec. Prog. Horm. Res., 40:441-480.

Foxcroft, G.R., Haresign, N.B.,Haynes, N.B., Lamming, G.E., Peters, A.R. (1988). Gonadotropins-Domestic Animals. Acta Endocrinol., 288:41-50.

Foxcroft, G.R. (1992). Nutritional and lactational regulation of fertility in sow. J. Reprod. Fertil. Suppl. 45:112-125

Gregg, D.W., Moss, G.E., Hudgens, R.E., Malven, P.V. (1986). Endogenous opioid modulation of luteinizing hormone and prolactin in post-partum ewes and cows. J. Anim. Sci., 63:838-847.

Grossman, A. (1989). Opioid peptides, prolactin, and gonadotrophin regulation in the human. In: Brain opioid system in reproduction. Eds Dyer, R.G., Bicknell, R.J. Oxford University Press, 325-339.

Ebling, F.J.P., Lincoln, G.A., Martin, G.B., Taylor, P.L. (1987). LHRH and beta-endorphin in the hypothalamus of the ram in relation to photoperiod and reproductive activity. Dom. Anim. Endocrinol., 4:149-156.

Haynes, N.B., Lamming, G.E., Yang, K.P., Brooks, A.N., Finnie, A.D. (1989). Endogenous opioid peptides and farm animal reproduction. In: Oxford Reviews of Reproductive Biology. Ed. S.R. Millingan, 11:111-145.

Kalra, S.P., Kalra, P.S. (1984). Opioid-adrenergic-steroid connection in regulation of luteinizing hormone secretion in the rat. Neuroendocrinol., 38:418-426.

Knight, P.G., Howles, C.M., Cunningham, F.J. (1986). Evidence that opioid peptides and dopamine participate in the suckling-induced release of prolactin in the ewe. Neuroendocrinol., 44:29-35.

Matteri, R.L., Moberg, G.P., (1985). The effect of opioid peptides on ovine pituitary gonadotropin secretion in vitro. Peptides, 6:957-963.

Mattioli, M., Conte, F., Seren, E., Galeati, G. (1986). Effect of naloxone on plasma concentrations of prolactin and LH in lactating sows. J. Reprod. Fertil., 76:167-173.

Myers, T.R., Myers, D.A., Gregg, D.W., Moss, G.E. (1989). Endogenous opioid release of luteinizing hormone during suckling in post-partum anestrous beef cows. Dom. Anim. Endocrinol., 6:183-190.

Owens, D.P., Cicero, T.J., (1981). Development of acute tolerance to the effects of naloxone on the hypothalamic-pituitary-luteinizing hormone axis in the male rat. J. Pharmacol. exp. Ther., 216:135-141.

Rawlings, N.C., Churchill, I.J. Currie, W.D., Joseph, I.B. (1991). Maturational changes in opioidergic control of luteinizing hormone and follicle stimulating hormone in ram lambs. J. Reprod. Fertil., 93:1-7.

SAS. (1985). Sas User's Guide: Statistics Cary, NC: Statistical Analysis System Institute, Inc., Cary.

Sesti, L.A., Britt, J.H. (1991) Agonist-induced release of GnRH, LH and FSH and their association with basal secretion of LH and FSH in lactating sow. Biol. Reprod., Suppl. 1, Abstract 209.

Sesti, L.A., Britt, J.H. (1993). Influence of stage of lactation, exogenous LHRH, and suckling on estrus, positive feed-back of LH, and ovulation in sow treated with estrogen. J. Anim. Sci., 71:989-998.

Shaw, H.J., Foxcroft, G.R. (1985). Relationship between LH, FSH, and prolactin secretion and reproductive activity in the weaned sow. J. Reprod. Fertil., 75:17-28.

Smith, C.A, Almond, G.W, Dial, G.D. (1992). Changes in luteinizing hormone concentrations after abortion, parturition, and weaning in primiparous sows. Anim. Reprod. Sci., 27:169-182.

Tokach, M.D., Pettigrew, J.E., Dial, G.D., Wheaton, J.E., Crooker, B.A., Johnston, L.J. (1992). Characterization of LH secretion in the primiparous lactating sow:relationship to blood metabolites and return to estrus interval. J. Anim. Sci. 70:2195-2201.

Varley, M.A, Foxcroft, G.R. (1990). Endocrinology of the lactating and weaned sow. J. Reprod. Fertil., Suppl., 40: 47-61.

Whisnant, C.S., Kiser, R.E., Thompson, F.N., Barb, C.R. (1986b). Naloxone inhibition of luteinizing hormone secretion during the post-partum period in suckled beef cows. J. Anim. Sci., 63:1445-1448.

Wu, T., McHartur, N.H., Harms, P.G. (1992). A possible dual mechanism involved in the inhibitory influence of suckling on luteinizing hormone release in the ovariectomized rat. Biol. Reprod., 46:653-657

# CHAPTER 4

## EXPERIMENT 3: INTERACTION BETWEEN SUCKLING AND MORPHINE ADMINISTRATION IN THE REGULATION OF LH AND PROLACTIN SECRETION IN THE SOW.

### 4.1 Introduction

Endogenous opioid peptides (EOP) have been implicated as potential modulators of gonadotropin secretion in domestic animals (Mattioli et al., 1986; Barb et al., 1986; Rawlings and Churchill, 1990; Ebling et al., 1985, 1989; Schillo, 1993; Rawlings et al., 1991; Armstrong et al., 1988b; De Rensis et al., 1983c). In the pig, the opioid antagonist (naloxone) blocks the inhibitory effect of suckling on LH secretion in mid- and late-lactation (Mattioli et al., 1986; Barb et al., 1986; De Rensis et al., 1993). Also, Armstrong et al. (1988a) demonstrated that the administration of morphine prevented the increase in LH associated with transient weaning and delayed the onset of estrus after weaning.

In previous work (De Rensis et al., 1993b) on suckling-induced regulation of LH secretion in early lactation, suckled sows were observed to have relatively high plasma LH concentrations from parturition until approximately 45-55 hours post-partum. LH then decreased to low levels typical of established lactation. When sows were Zero-Weaned, by removing all piglets immediately after farrowing and thus the suckling induced inhibition of LH, LH secretion remained high and was characterized by a high frequency episodic-type release.

In later studies (De Rensis et al., 1993c) and Experiment 2 of this thesis, chronic naloxone treatment was not able to reverse the suckling-induced inhibition of LH secretion in the early post-partum period but an effect was evident at day 10 of lactation. Also, PRL secretion was not modified during the early post-partum period by naloxone but naloxone induced a decrease in PRL at day 10 and 11 of lactation. These data indicate that there are temporal differences in the effect of endogenous opioids on LH and PRL secretion during lactation.

A number of specific conclusions were drawn from these earlier studies. 1) Opioid inhibition may not occur during the early post-partum period; 2) effects of endogenous opioid peptides may be masked by an alternative inhibitory system and 3) if present, opioid receptors present may not readily be antagonized by naloxone.

Multiple opioid receptor types have been implicated in suppression of LH secretion but it is generally believed that the mu receptor subtype is most involved (Pfeifer et al., 1983; Panerai et al., 1985). Morphine and naloxone do not have identical affinities for all opioid receptor types. Both bind with high affinity to mu1- and mu2-receptors (Lord et al., 1977; Casy et al., 1986; Pasternak et al., 1986a,b; Kazmi et al., 1987) but naloxone also binds to delta-and kappa receptors (Khatchaturian et al., 1985; Chang., 1984). It has also been demonstrated that morphine inhibits the firing frequency of hypothalamic neurones associated with the release of LHRH (Kesner et al., 1986).

Therefore, the aim of the present experiment was to investigate whether the administration of morphine, an opioid agonist, would modify LH secretion during early lactation. Furthermore, to investigate the role of the opioidergic system in mediating the suckling-induced inhibitory effects on LH secretion, morphine was administered to sows whose piglets were removed immediately after farrowing, as well as to sows nursing their piglets.

## 4.2 MATERIALS AND METHODS

### 4.2.1 Animals and blood collection

A total of 19 primiparous sows (Camborough; Pig Improvement (Canada) Ltd) were used in a replicated experiment. Sow were housed in conventional farrowing crates and water and feed were provided twice a day throughout lactation. Within each replicate (....and.... sows, respectively), sows were randomly allocated to two groups: In a total of nine "Zero-Weaned" sows, all piglets were removed from each sow 6h after the birth of the last piglet. This procedure allows new born piglets to obtain an immediate supply of colostrum from their dams prior to fostering to other sows: A total of ten "Suckled" sows (five replicate 1, five replicate 2), suckled 10 or more piglets for the duration of the trial. To facilitate the logistics of the experiment, farrowing times were grouped in 12h blocks and time zero was taken as the end of each 12h period. The sows were fitted with indwelling jugular cannulae via the cephalic vein under general anaesthesia 12-18 h post-partum to allow stress-free frequent blood sampling. Plasma was stored at -20°C until analysis of all samples for LH and prolactin (PRL) concentrations by radioimmunoassay.

Blood samples were collected at 10 min intervals during four sampling periods, 24-30h, 48-54h, 72-78h post-partum, and for 12h on day 10 of lactation. In a previous dose-response study we determined that a dose of 0.1mg/kg of morphine did not induce any adverse behavioral side effects. Therefore in all sows, 0.1mg/kg of morphine was administered as three i.v. bolus injections at hourly intervals during the last 3h of each sampling period, and at 6, 7 and 8h after the beginning of sampling on day 10.

### 4.2.2 Hormone assays

Plasma LH concentrations in all samples were determined using the double antibody RIA described by De Rensis et al. (1983b). The intra- and inter-assay c.v. were 9.9% and 17.1%, respectively and the sensitivity of the assay, defined as 90% of total binding, was 0.13 ng/ml.

Plasma concentrations of PRL were measured by the method described by Shaw and Foxcroft (1985) with minor modifications. Intra- and inter-assay c.v. were 9.1 and 9.7%, respectively and the overall sensitivity of the assay defined as 85% of total binding was 1.9 ng/ml.

### 4.2.3 Statistical analysis

Subjective assessment of the plasma LH profiles obtained in this study indicated that the adoption of computerized pulse analysis programs was inappropriate (for details see Foxcroft et al., 1988). Therefore to provide mean characteristics of LH secretion, LH profiles were analyzed by the method of Shaw and Foxcroft (1985) using a sliding window technique.

The 6h LH and PRL profiles for the first three sampling periods (days) were divided to generate means for two 3h periods (before and after morphine injections). Hormone profiles from the 12h frequent-sampling period at day 10 were analyzed as means for two 6h periods. A repeated measures analysis of variance was used to test for the effects of morphine within days and the effect of days. Main effects of sow group (Zero-Weaned versus Suckled) and experimental replicate (first or second), and interactions between main effects and repeated measures, were fitted. When significant interactions between main effects were established, relevant main effect least-square means were compared by Student's t test. Polynomial contrasts were used to evaluate a linear, quadratic and cubic trends for time.

## 4.3 RESULTS

LH

The individual patterns of LH secretion in four Zero-Weaned and four Suckled sows are shown in Fig. 4.1-4.4. Overall mean (+SEM) plasma concentrations of LH during the four sampling periods are shown in Fig.4.5. Significant effects of group (Zero-Weaned versus Suckled; $p<0.001$), day ($p<0.001$) and a day x treatment x group interaction ($p<0.001$) were established. Zero-Weaned sows exhibited higher LH concentrations ($p<0.001$) during all except the first day of sampling. Use of polynomial contrasts to elucidate interactions between repeat measures established a quadratic ($p<0.001$) increase in LH throughout the post-partum period in Zero-Weaned sows in the absence of morphine compared to a significant ($P<0.05$) linear relationship in the absence of morphine. A linear ($p<0.05$) increase was observed in Suckled sows over the same period in the presence of morphine treatment but no time dependent change in LH secretion was established in the absence of morphine.,

PRL

The individual patterns of PRL secretion in two Zero-Weaned and two Suckled sows are shown in Fig. 4.6-4.9 and overall mean (+SEM) plasma concentrations of PRL during the four sampling periods are shown in Fig. 4.10. There was a significant ($p<0.001$) group effect, and morphine x group interaction on plasma PRL within suckled sows. There was a morphine effect ($p<0.001$) but not a morphine x day interaction. In Suckled sows, plasma PRL was already high at the beginning of sampling and did not change with time. In this group morphine decreased ($p<0.05$) mean plasma PRL. In Zero-Weaned sows, plasma PRL was already low at the beginning of sampling and did not change with time. No effects of morphine were observed in this group.

## 4.4 DISCUSSION

LH

In this study the exact time of suckling bouts was not recorded because of the period of lactation involved. During the very early post-partum period the piglets spent a considerable amount of time attached to the teat and it is difficult to exactly distinguish discrete suckling activity. However, casual observations during the experiment indicated that the piglets nursed throughout all the experimental period.

As in previous studies, the pattern of LH secretion did not allow us to describe precise LH pulse characteristics due to the high-frequency, variable-amplitude, episodic-like release of LH. However, a significant difference in mean plasma LH levels between Zero-Weaned and Suckled sows was observed beginning 48h post-partum. These observations are in agreement with our previous studies (De Rensis et al., 1993b) and further confirm that the suckling stimulus is the main factor blocking LH secretion during lactation in the sow. These observations in part differ from the recent study of Smith et al. (1992) who reported that mean LH concentrations differed between Zero-Weaned sows and Suckled sows only after day 14 of lactation. However, in their data LH pulse frequency was already different between the two groups at day 4.

In contrast to our own previous work (De Rensis et al., 1983b) and to other studies in suckled rats (Dondi et al., 1991) and sows (Tokach et al., 1992), a clear, time-dependent, suckling-induced inhibition of LH was not observed. This could be due to the experimental protocol utilized in the present study which did not include sampling immediately after farrowing, or to the particular animals utilized in this experiment. Whenever the cause, LH secretion was already inhibited at the time of the first sampling period.

With regard to the neuroendocrine control of gonadotropin secretion, it is accepted that one of the mechanisms by which the suckling stimulus inhibits LH secretion during lactation is mediated via the opioidergic system. In fact, several studies describe that treatment with opioid antagonists modulates LH secretion during lactation in the sow (for review see Barb et al., 1991; De Rensis et al., 1993c; Britt et al., 1993). In this study, morphine decreased LH secretion in Suckled sows with a more evident effect at day 10 of lactation, and in Zero-Weaned sows during sampling 72-78h post-partum and at day 10 post-partum compared to the previous sampling periods. The presence of an effect of morphine on LH secretion in Suckled sows in mid-lactation, when LH secretion is very low, is particulary interesting. Armstrong et al. (1988a) also observed a decrease in LH secretion after morphine infusion in some sows at day 25 of lactation but, overall, these authors did not observe a significant effect of morphine on LH secretion in late lactating sows. The fact that morphine significantly inhibited LH secretion in our study could be related to the different protocol used for morphine administration or to the larger number of animals utilized in our experiment. However, a different sensitivity to morphine among animals could be another factor. Indeed, there are several studies reporting that large variability in the sensitivity to opioid agonists and antagonists exists between animals (Armstrong et al., 1986; Barb et al., 1991; De Rensis et al., 1993c,).

Several studies report that opioids effects on LH may involve mu and kappa receptors (Goodman et al., 1980; Cicero et al., 1983; Pfeiffer et al., 1983; Panerai et al., 1985; Laedem et al., 1985). However, the studies of Weisner et al. (1985) indicate that delta but not mu receptors are implicated in opioid inhibition of LH. The results of the present study indicate that receptors sensitive to morphine are not fully occupied during established lactation in the sow and treatment with an exogenous opioid agonist is able to effect an even greater inhibition of LH secretion.

## PRL

Similar to our own previous studies (De Rensis et al., 1993a,b) and other data (Stevenson et al., 1981), prolactin concentrations in Suckled sows were high soon after farrowing. In our study, a gradual but not significant decline in plasma PRL was observed with time during lactation, again confirming data from previous experiments (De Rensis et al., 1993b). In Zero-Weaned sows plasma PRL was already low at the time of the first period of sampling and did not change during the later part of the experiment. These differences between groups in PRL secretion confirms that the suckling stimulus is an essential part of the mechanism that regulates prolactin secretion after farrowing.

The observation of an inhibitory effect of morphine on PRL secretion in Suckled sows was unexpected. Others have reported a stimulatory effect of opioids on prolactin (Bruni et al., 1977; Shaar et al., 1980; Schillo, 1985) and morphine injection in the post-partum cow and steer increased PRL secretion (Peck et al., 1988a,b). Finally, naloxone, an opioid antagonist, decreased prolactin in lactating sows (Mattioli et al., 1986; Armstrong et al., 1988b; De Rensis et al., 1993). However, in one previous study it was observed that morphine did not increase, but suppressed prolactin (Armstrong et al., 1988a) in lactating sows. These authors suggested that this was due to the dose, or the duration of morphine treatment, or to previous exposure to high levels of endogenous opioids during parturition. Furthermore, in lactating rats, morphine administration (Callahan et al., 1988) did not induce an increase in prolactin levels; this refractoriness to morphine stimulation did not appear to be due to an alteration in drug metabolism, since there were no increases in prolactin levels regardless of the route of administration.

There are also studies in the human that indicate that elevated levels of prolactin in the immediate puerperium are not affected by naloxone (Grossman et al., 1982; Lodico et al., 1983). Lodico et al. (1983) and Cholst et al. (1984) showed that the prolactin response to breast stimulation is not blunted by naloxone infusion and is probably not mediated by an endogenous opioid pathway.

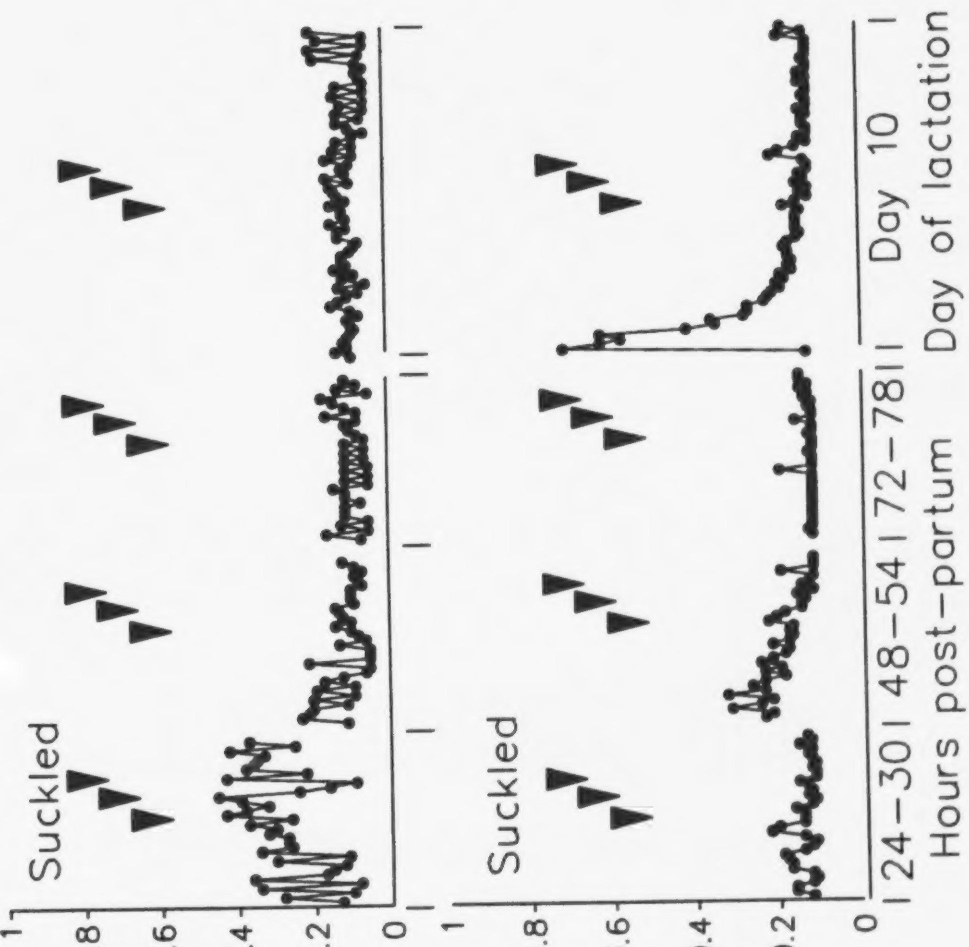
Our own study and that of Armstrong et al. (1988) indicate that morphine decreases PRL secretion during lactation in the sow. One possible explanation for this effect of morphine may be the existence of separate and distinct opiate receptor subtypes. As for LH, there are discordancies regarding the opioids receptors involved in the control of PRL. In fact it is generally believed that opiate receptors involved in prolactin secretion belong to the mu (Rossi et al., 1983; Akil et al., 1984; Koening et al., 1984) and kappa classes (Zuckin et al., 1981). However, Koening et al. (1984) have reported that the beta-endorphin effect on prolactin is not blocked by a mu1-antagonist, suggesting that this site is not involved in mediating the stimulatory effect of this peptide on prolactin. Therefore, in contrast to a general stimulatory effect of opioids on prolactin secretion (see Ben-Jonathan et al., 1989), morphine administration may instead stimulate neuroendocrine mechanisms that inhibit prolactin secretion.

## 4.5 CONCLUSION

In conclusion, this study confirms that the suckling stimulus is associated with suppressed plasma LH and elevated plasma PRL concentrations. Furthermore, data from this study are consistent with a time dependent development of endogenous opioid peptide inhibition of LH secretion. During the early post-partum period the opioidergic system may require some time to become fully operative or the effects of endogenous opioid peptide antagonism may be masked by an alternative inhibitory system. Finally, the presence of different opioid receptor populations cannot be excluded.

The presence of an inhibitory effect of morphine on prolactin secretion during lactation, in the presence of the suckling stimulus, suggests that opiate receptors sensitive to morphine are not involved in the regulation of prolactin secretion during lactation or that morphine is stimulating the activity of systems that have an inhibitory effect on PRL secretion.

**Fig. 4.1** Individual plasma LH profiles in two Suckled sows. Arrows indicate morphine injections.



Suckled
Suckled
LH(ng/ml)
LH (ng/ml)
1
0.8
0.6
0.4
0.2
0
24–30
48–54
72–78
Day 10
Hours post-partum
Day of lactation

**Fig. 4.2** Individual plasma LH profiles in two Suckled sows. Arrows indicate morphine injections

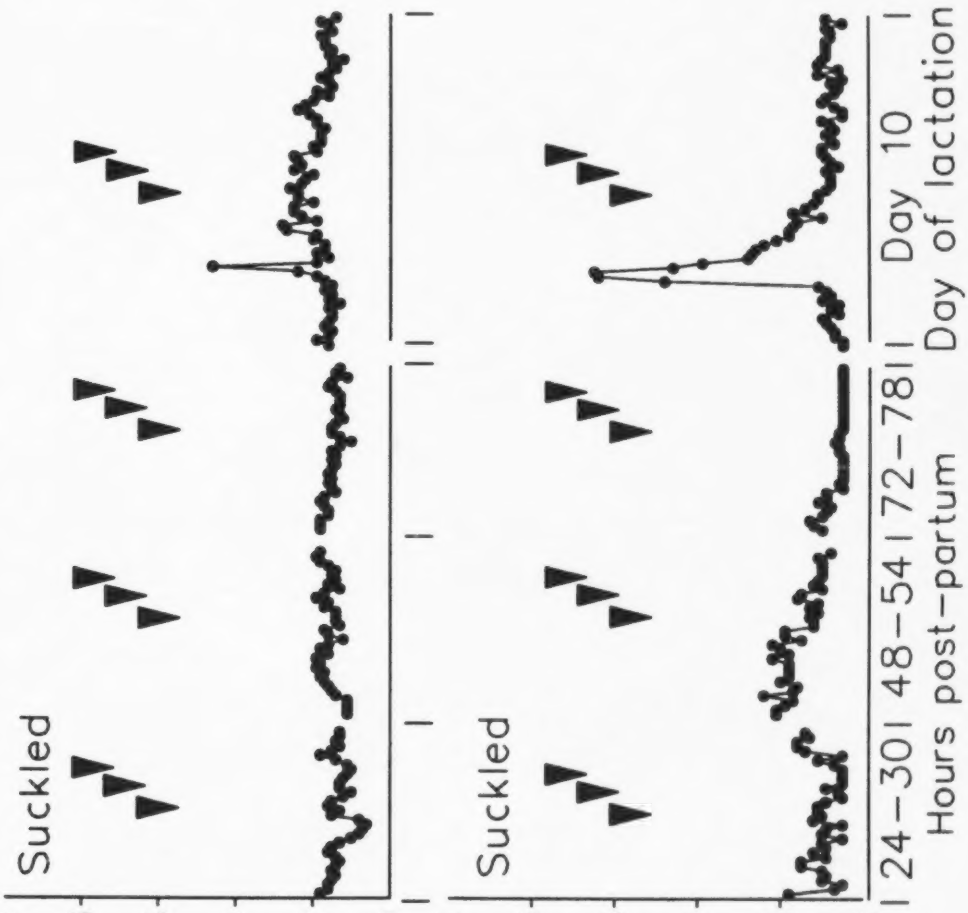

Suckled
LH(ng/ml)
0
0.2
0.4
0.6
0.8
1
Suckled
LH (ng/ml)
0
0.2
0.4
0.6
0.8
1
24–30
48–54
72–78
Hours post-partum
Day 10
Day of lactation

**Fig. 4.3** Individual plasma LH profiles in two Zero-Weaned sows (piglets were removed immediately after farrowing). Arrows indicate morphine injections.

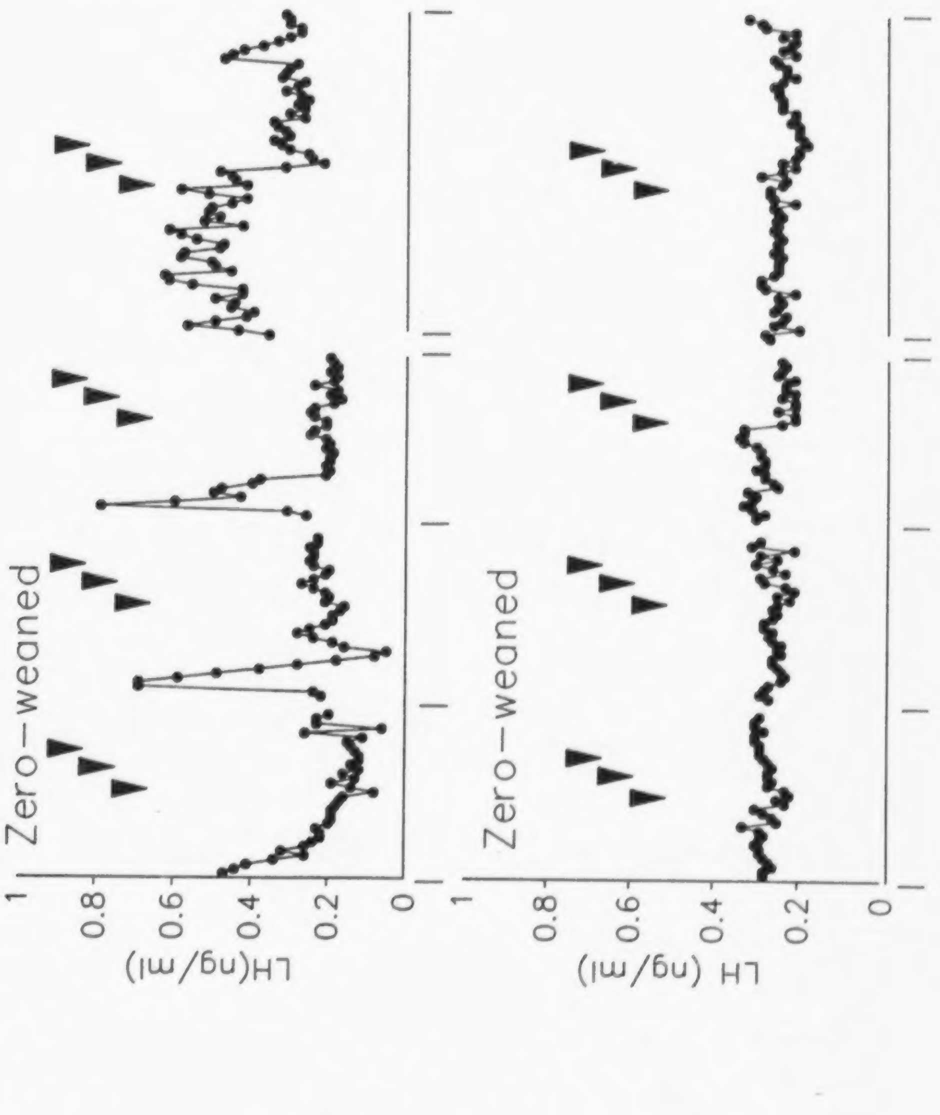
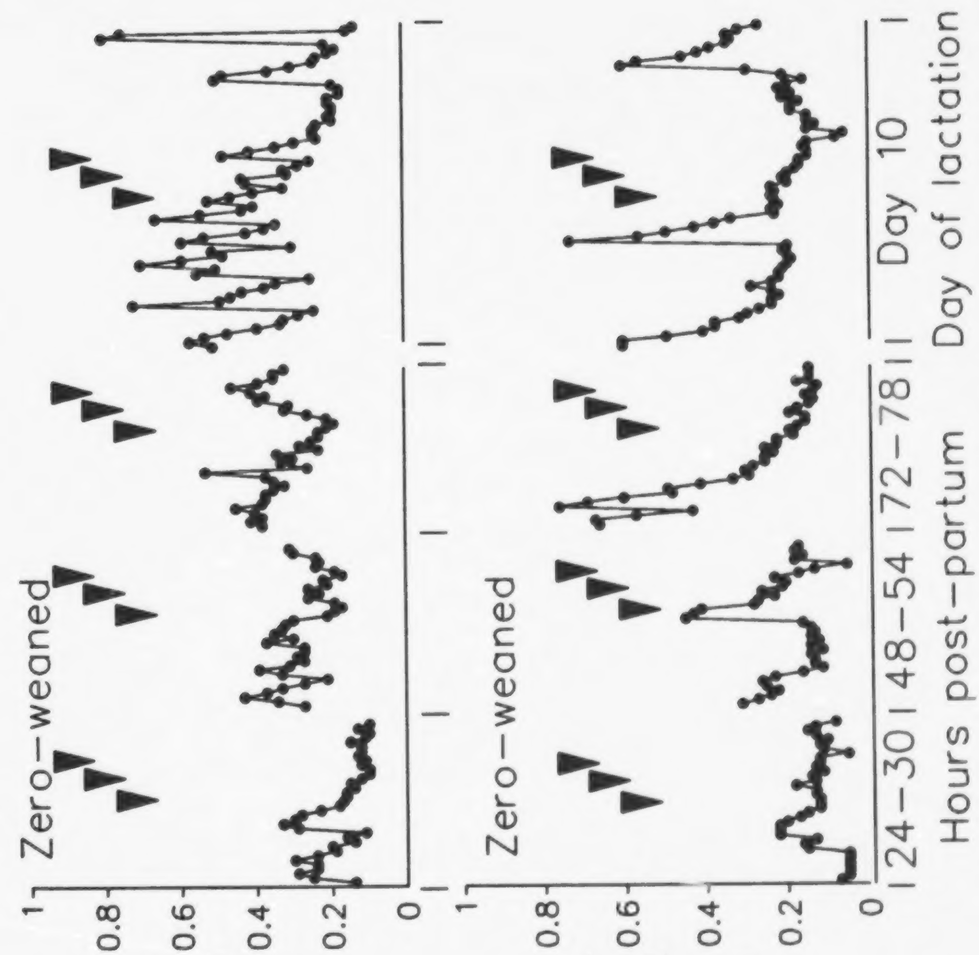
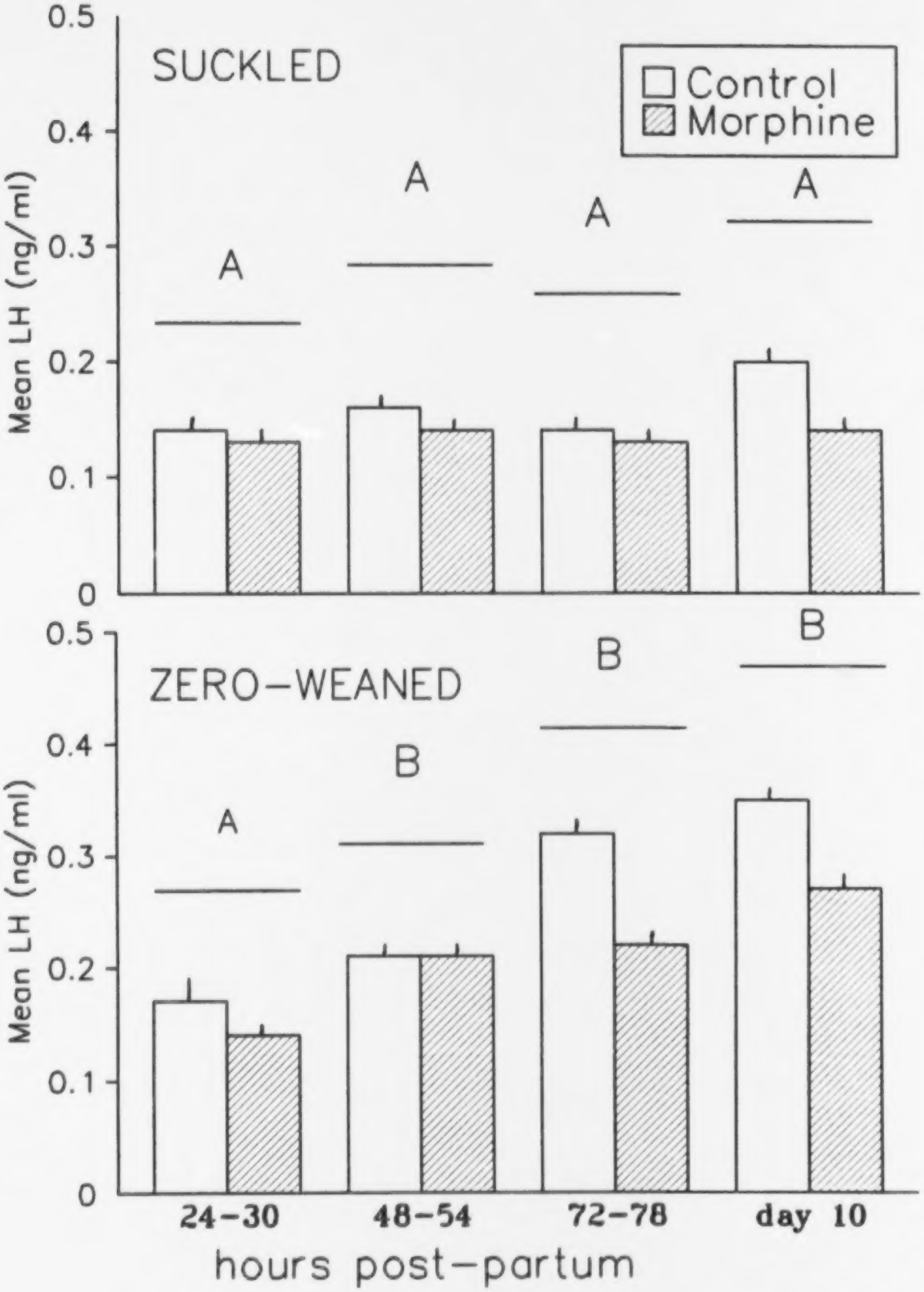

Zero-weaned
LH(ng/ml)
1
0.8
0.6
0.4
0.2
0
Zero-weaned
LH (ng/ml)
1
0.8
0.6
0.4
0.2
0

**Fig. 4.4** Individual plasma LH profiles in two Zero-Weaned sows (piglets were removed immediately after farrowing). Arrows indicate morphine injections.



Zero-weaned
LH(ng/ml)
1
0.8
0.6
0.4
0.2
0
Zero-weaned
LH (ng/ml)
1
0.8
0.6
0.4
0.2
0
24-30
48-54
72-78
Day 1
Day 10
Hours post-partum
Day of lactation

**Fig. 4.5** Mean (+SEM) plasma LH. Each bar indicates the mean for 3h-periods of sampling. Means with different superscripts differ between groups irrespective of morphine treatment and confirm a suppressive effect of suckling on LH secretion within 54h of farrowing. Morphine treatment attenuated the trend for an increase in mean plasma LH in both groups (see results).



SUCKLED
ZERO-WEANED
Control
Morphine
Mean LH (ng/ml)
0
0.1
0.2
0.3
0.4
0.5
A
A
A
A
A
B
B
B
24-30
48-54
72-78
day 10
hours post-partum

**Fig. 4.6** Individual plasma PRL profiles in two Suckled sows. Arrows indicate morphine injections.

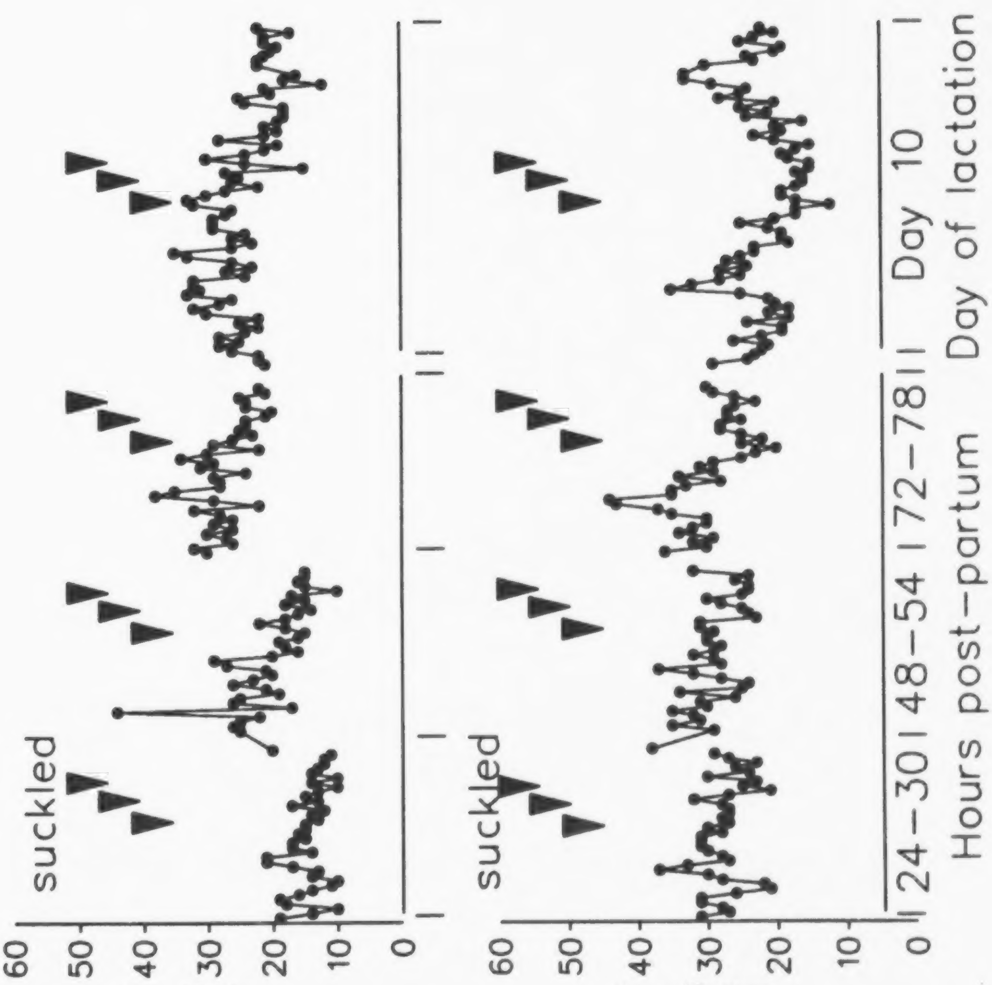
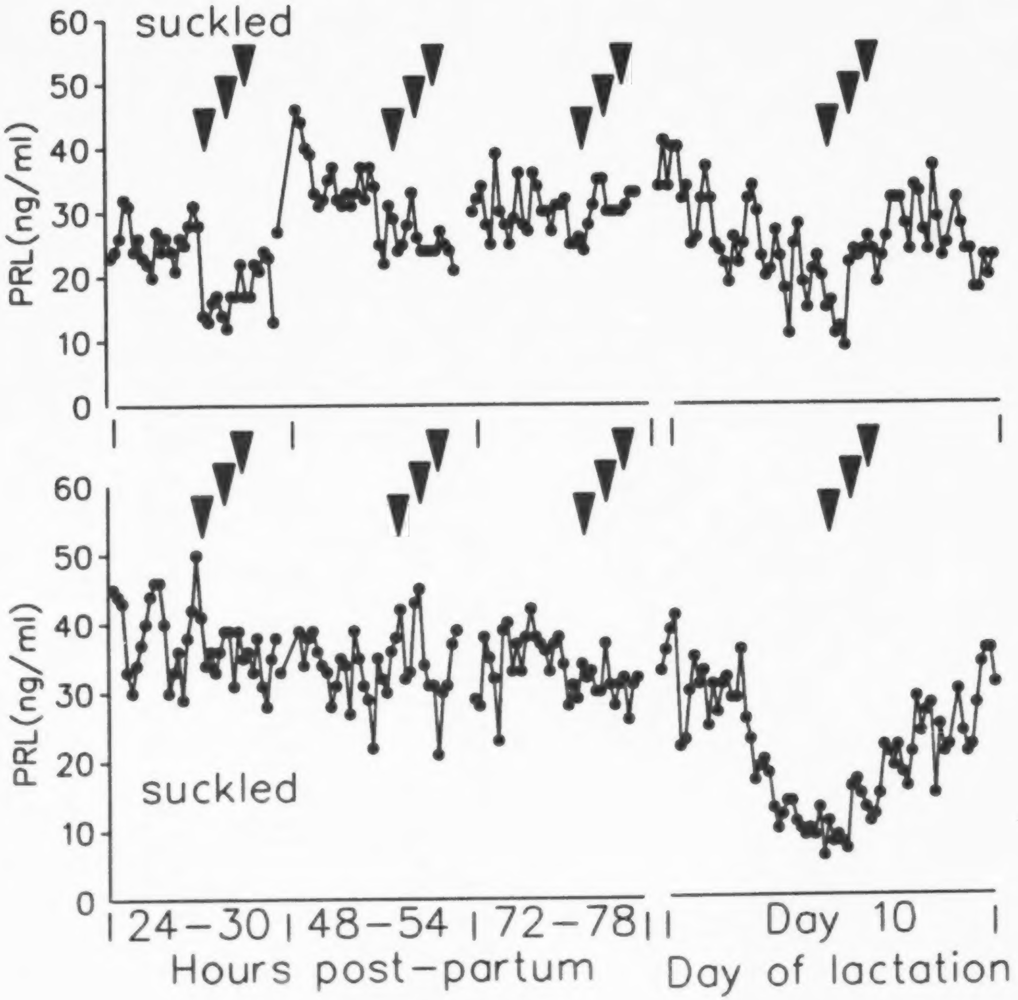

PRL(ng/ml)
0
10
20
30
40
50
60
suckled
suckled
24–30
48–54
72–78
Day 10
Hours post-partum
Day of lactation

**Fig. 4.7** Individual plasma PRL profiles in two Suckled sows. Arrows indicate morphine injections.



suckled
PRL(ng/ml)
60
50
40
30
20
10
0
suckled
PRL(ng/ml)
60
50
40
30
20
10
0
24-30
48-54
72-78
Hours post-partum
Day 10
Day of lactation

**Fig. 4.8** Individual plasma PRL profiles in two Zero-Weaned sows (piglets were removed 6h after farrowing). Arrows indicate morphine injections.

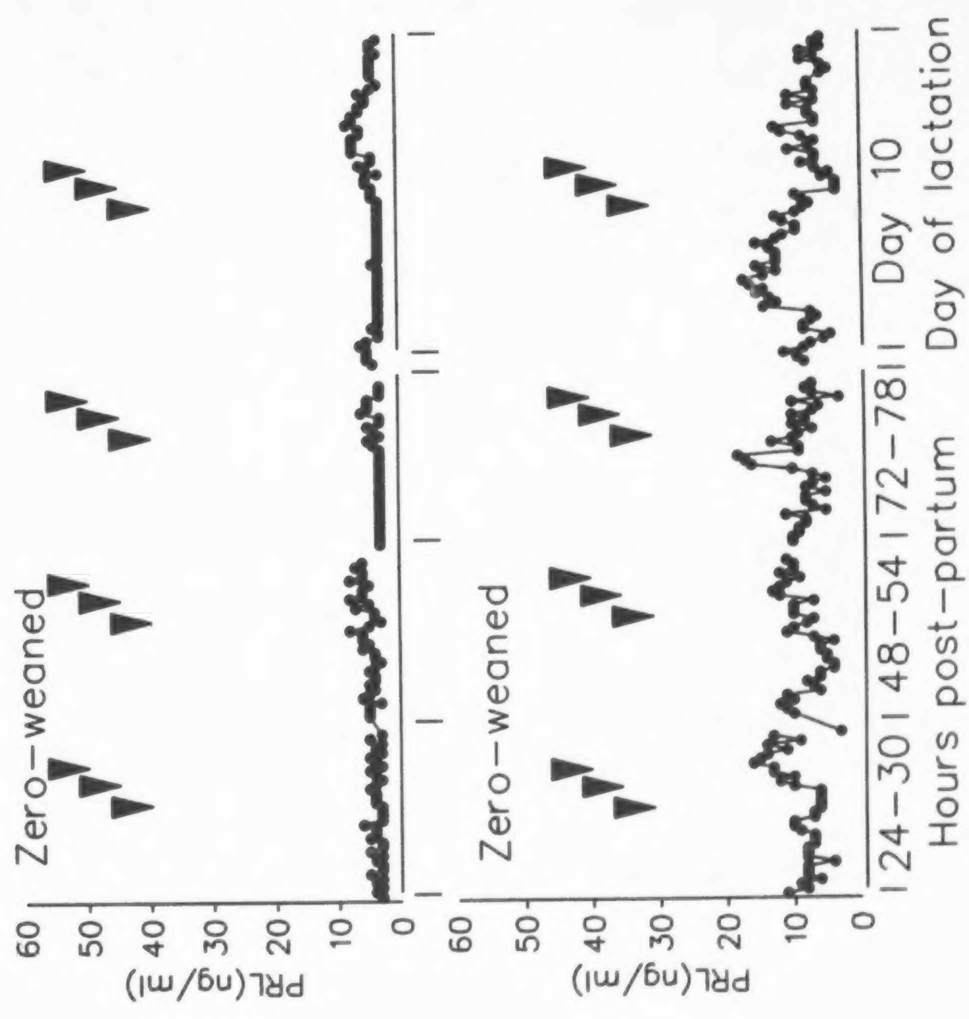

Zero-weaned
Zero-weaned
PRL (ng/ml)
PRL (ng/ml)
60
50
40
30
20
10
0
24-30
48-54
72-78
Day 10
Hours post-partum
Day of lactation

**Fig. 4.9** Individual plasma PRL profiles in two Zero-Weaned sows (piglets were removed 6h after farrowing). Arrows indicate morphine injections.

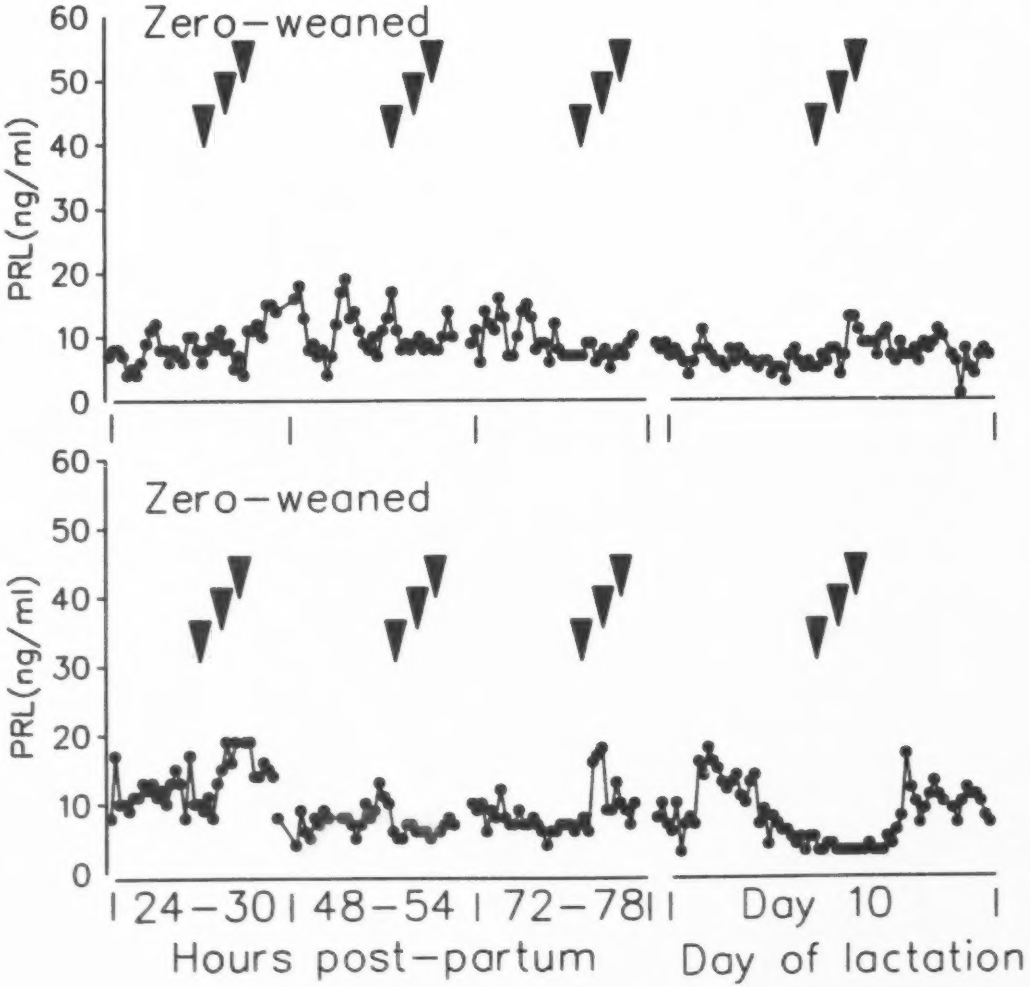
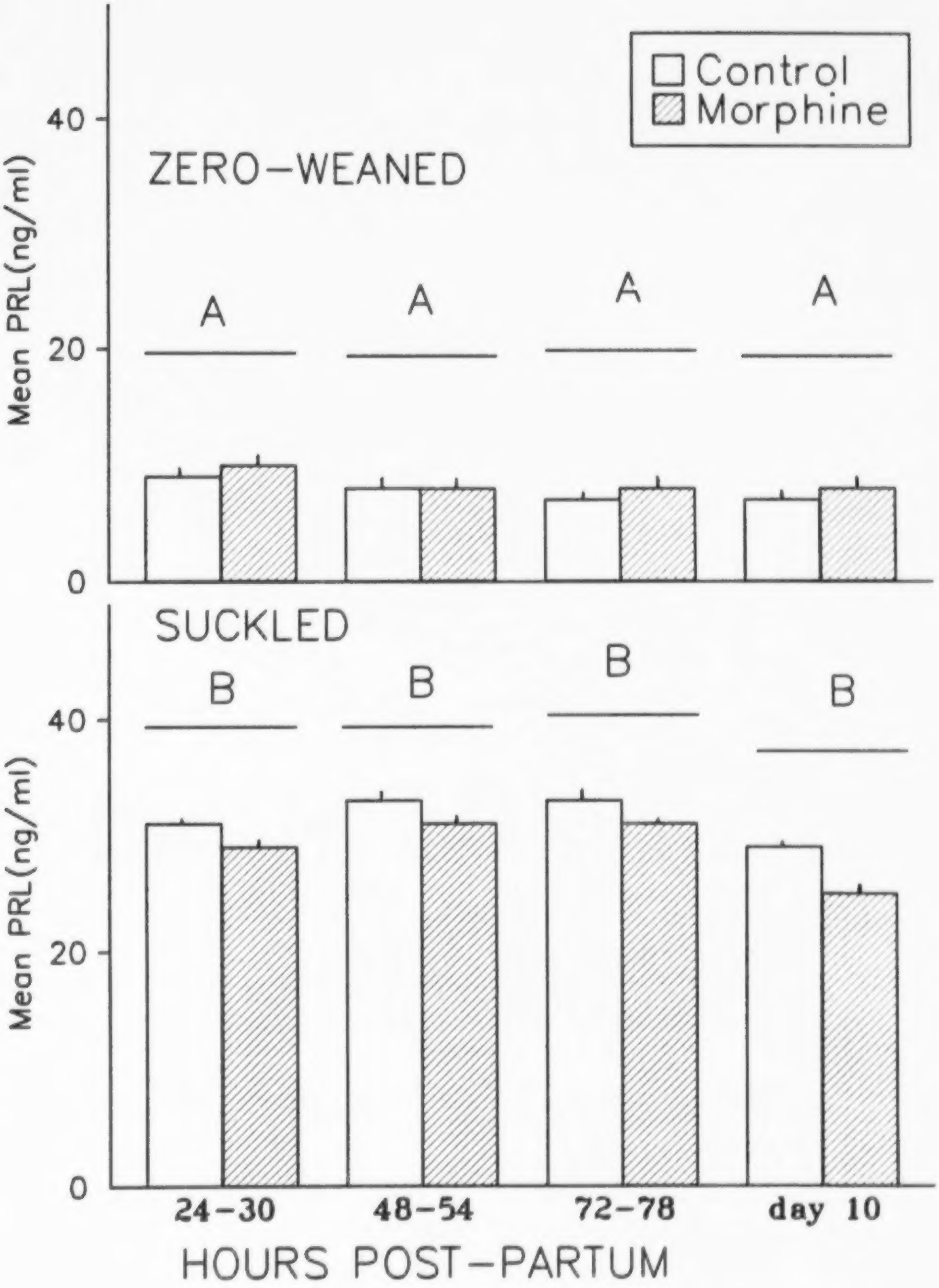

Zero-weaned
PRL(ng/ml)
60
50
40
30
20
10
0
Zero-weaned
PRL(ng/ml)
60
50
40
30
20
10
0
24–30
48–54
72–78
Day 10
Hours post-partum
Day of lactation

**Fig. 4.10** Mean (+SEM) plasma PRL. Each bar indicates the mean for 3h-periods of sampling. Means with different superscripts differ between groups irrespective of morphine treatment and confirm the immediate and sustained influence of suckling on PRL secretion. Overall in Suckled sows morphine decreased ($p<0.05$) mean plasma PRL. No effects of morphine were observed in Zero-Weaned sows.



☐ Control
▨ Morphine
ZERO-WEANED
SUCKLED
A
A
A
A
B
B
B
B
Mean PRL (ng/ml)
Mean PRL (ng/ml)
40
20
0
40
20
0
24-30
48-54
72-78
day 10
HOURS POST-PARTUM

## 4.6 BIBLIOGRAPHY

Akil, H., Warson, S.J., Young, E., Lewis, M.E., Khachaturian, H., Walker, J.M. (1984). Endogenous opioids, biology and function. Ann. Rev. Neurosci., 7:223.

Armstrong, J.D., Kraeling, R.R., Britt, J.H. (1986). Effect of bolus administration of naloxone on secretion of LH and prolactin in lactating sows. J. Anim. Sci., (Suppl. 1) Abstract 322.

Armstrong, J.D., Kraeling, R.R. and Britt, J.H. (1988a). Morphine suppresses luteinizing hormone concentrations in transiently weaned sows and delays onset of oestrus after weaning. J. Anim. Sci., 66:2216-2222.

Armstrong, J.D., Kraeling, R.R, Britt, J.H. (1988b). Effects of naloxone or transient weaning on secretion of LH and prolactin in lactating sows. J. Reprod. Fertil., 83:301-308.

Barb, C.R., Kraeling, R.R., Rampacek, G.B. and Whisnant, C.S. (1986). Opioid inhibition of luteinizing hormone secretion in the postpartum lactating sow. Biol. Reprod. 35:368-371.

Barb, C.R., Rampacek, G.B., Kraeling, R.R., Estienne, M.J., Taras, E., Estienne, C.E. and Whisnant, C.S. (1988). Absence of brain opioid peptide modulation of luteinizing hormone secretion in the prepubertal gilt. Biol. Reprod., 39:603-609.

Barb, C.R., Kraeling, R.R., Rampacek, G.B. (1991). Opioid modulation of gonadotropin and prolactin secretion in domestic farm animals. Dom. Anim. Endocrinol., 91:15-27.

Ben-Jonathan, N., Arbogast, L.A., James, F.H. (1989). Neuroendocrine regulation of prolactin release. Prog. Neurobiol., 33:399-447.

Britt, J.H. Armstrong, J.D. Moore, K.L. Sesti, L.A.C. (1993). Involvement of opioids in regulation of LH secretion during lactational- or nutritional-induced anestrus in pig and cattle. In: Opioids in farm animals, Ed. N.Parvizi, Landwirtschaftsverlag GmbH, 4400 Münster-Hiltrup, Germany. pp. 34-54.

Bruni, J.F., Van Vugt, D.A., Marshall, S., Meites, J. (1977). Effects of naloxone, morphine and methionine on serum prolactin, luteinizing hormone, follicle stimulating hormone and growth hormone. Life Sci., 21:461.

Callahan, P., Janik, J., Grandison, L., Rabii, J. (1988). Morphine does not stimulate prolactin release during lactation. Brain Research, 442:214-222.

Casy, A.F., Parfitt, R.T. (1986). Opioid Analgesics, chemistry and receptors. In: Opioid Analgesics. Plenum Press, New York, pp. 333-337.

Chang, K.J. (1984). Opioid receptors: Multiplicity and Sequelae of ligand-receptor interactions. In: The receptors. Ed. P.M. Conn. Academic Press, New York, 1:1-81.

Cholst, I.N., Wardlaw, S.L., Connie, B., Newman, C.B. and Franz, A.G. (1984). Prolactin response to breast stimulation in lactating women is not mediated by endogenous opioids. Am. J. Obstet. Gynecol., 150:558-561.

Cicero, T.J., Owens, D.P., Schmoeker, P.F., Meyer, E.R. (1983). Morphine induced enhancement of the effect of naloxone on serum luteinizing hormone levels in male rat: specificity for mu antagonists. J. Pharmacol. exp. Ther., 226:770-774.

Crowley, W.R., (1988) Role of endogenous opioid neuropeptides in the physiological regulation of luteinizing hormone and prolactin secretion. In: Peptide hormones, effect and mechanisms of action. Eds. Negro-Vilar, A., Conn, P.M. , C.R.S. Press, 3: 79-116.

Currie, W.D. and Rawlings, N.C. (1989). Fluctuation of responsiveness of LH and lack of responsiveness of FSH to prolonged infusion of morphine and naloxone in the ewe. J. Reprod. Fert., 86:359-366.

De Rensis, F. Foxcroft, G.R. (1993a). Endogenous opioid modulation of gonadotropin and prolactin secretion in the post-partum sow. In: Opioids in farm animals, Ed. N.Parvizi, Landwirtschaftsverlag GmbH, 4400 Münster-Hiltrup, Germany. pp. 15-33.

De Rensis, F., Hunter, M.G., Foxcroft, G.R. (1993b). Suckling-induced inhibition of LH secretion and follicular development in the early post-partum sow. Biol Reprod., 48:964-969.

De Rensis, F. Cosgrove, J.R., Foxcroft, G.R. (1993c). Luteinizing hormone and prolactin responses to naloxone vary with stage of lactation in the sow. Biol Reprod., 48:970-976.

Dondi, D., Maggi, R., Panerai, A., Piva, F., Limonta, P. (1991). Hypothalamic opiatergic tone during pregnancy, parturition and lactation in the rat. Neuroendocrinol., 53:460-466.

Ebling, F.J.P., Lincoln, G.A. (1985). Endogenous opioids and the control of seasonal LH secretion in Soay rams. J. Endocrinol., 107:341-353.

Ebling, F.J.P., Schwartz, M.L., Foster, D.L. (1989). Endogenous opioid regulation of pulsatile luteinizing hormone secretion during sexual maturation in the female sheep. Endocrinology., 125:369-383.

Foxcroft, G.R., Haresign, W., Haynes, N.B., Lamming, G.E., Peters, A.R. (1988).

Gonadotropins-Domestic Animals. Acta Endocrinol., 288:41-50.

Goodman, R.R., Snyder, S.H., Kuhar, M.J., Young, W.S. (1980). Differentiation of delta and mu opiate receptor localization by light microscopic autoradiography. Proc. Nat. Acad. Sci. USA, 77:6239.

Grossman, A., West, S.W., Evans, J., Rees, L.H., Besser, G.M. (1982). The role of opiates in the control of prolactin in the puerperium, and TSH in primary hypothyroidism. Clin. Endocrinol., 16:317-320.

Kazmi, S.M.I., Mishra, R.K. (1987). Comparative pharmacological proprieties and functional coupling of μ and δ opioid receptor sites in human blastoma SH-SY5Y calls. Mol. Pharmacol., 32:109-

Khatchturian, H., Allesi, N.E., Lewis, M.E., Munfakh, N., Fitzsimmon, M.D., Watson, S.J. (1985). Development of hypothalamic opioid neurons: A combined immunocytochemical and 3[H]-thymidine autoradiographic study. Neuropeptides, 4:477-480.

Kesner, J.S., Kaufman, J.M., Wilson, R.C., Kuroda, G., Knobil, E. (1986). The effect of morphine on the electrophysiological activity of the hypothalamic luteinizing hormone releasing hormone pulse generator in the rhesus monkey. Neuroendocrinol., 43:686-688.

Koening, I.J., Mayfield, M.A., McCann, S.M., Kruhlich, L. (1984). Differential role of the opioid mu and kappa receptors in the activation of prolactin and growth hormone secretion of morphine in male rat. Life Sci., 34:1829-1837.

Laedem, C.A., Kalra, S.P. (1985). Effects of endogenous opioid peptides and opiates on LH and prolactin secretion. Neuroendocrinol., 41:342-352.

Lord, J.A.H., Waterfeld, A.A., Hughes, J., Kosterlitz, H.W. (1977) Endogenous opioid peptide: multiple agonist and receptors. Nature, 267:495-499.

Lodico, G., Stoppelli, I., Delitala, G., Maioli, M., (1983). Effects of naloxone infusion on basal and breast stimulation induced prolactin secretion in puerperal women. Fert. Steril. 40:600-603.

Mattioli, M., Conte, F., Seren, E., Geleati, G. (1986). Effect of naloxone on plasma concentrations of prolactin and LH in lactating sows. J. Reprod. Fertil., 76:167-173.

Panerai, A., Petraglia, F., Sacerdote, P., Genazzani, A.R. (1985). Mainly mu opiate receptors are involved in LH and prolactin secretion. Endocrinology., 117:1096-1099.

Pasternak, G.W. (1986). Multiple mu opiate receptors: biochemical and pharmacological evidence for multiplicity. Bioch. Pharmacol., 35:361-364.

Peck, D.D., Thompson, F.N., Jernigan, A., Kiser, T.E. (1988a). Effect of morphine on serum gonadotropin concentration in post-partum beef cows. J. Anim. Sci., 66:2930.

Peck, D.D., Thompson, F.N., Stuedemann, A., Leshin, L.S., Kiser, T.E. (1988b). Evidence for endogenous opioid modulation of serum luteinizing hormone and prolactin in steer. J. Anim. Sci., 66:3197-3201.

Pfeiffer, D.G., Pfeiffer, A., Shimahigashi, K., Merriam, G.R., Loriaux, D.L. (1983). Predominant involvement of mu rather than delta or kappa opiate receptors in LH secretion. Peptides, 4:647-649.

Rossi, A., Disalle, E., Briatico, G., Arcari, G., De Castiglione, R., Perseo, G. (1983). Antinociceptive, prolactin releasing an intestinal motility inhibiting activities of dermorphin and analogues after subcutaneous administration in the rat. Peptides, 4:577-580.

Rawlings, N. C., Churchill, I.J. (1990). Effect of naloxone on gonadotrophin secretion at various stages of development in the ewe lamb. J. Reprod. Fertil., 89:503-509.

Rawlings, N.C., Churchill, I.J., Currie, W.D., Joseph, I.B. (1991). Maturational changes in opioidergic control of luteinizing hormone and follicle stimulating hormone in ram lambs. J. Reprod. Fertil., 93:1-7.

SAS. (1985). Sas User's Guide: Statistics Cary, NC: Statistical Analysis System Institute, Inc., Cary..

Sarkar, D.K., Yen, S.S. (1985). Hyperprolactinemia decreases the LHRH concentration in pituitary portal plasma: a possible role for beta-endorphin as mediator. Endocrinology., 116:2080-2084.

Schillo, K.K., Alliston, C.W., Malven, P.V. (1985). Do endogenous opioid peptides mediate the effects of photoperiod on release of luteinizing hormone and prolactin in ovariectomized ewes?. Biol. Reprod., 32:779-787.

Schillo, K.K. (1993). Possible roles of endogenous opioids in control of luteinizing hormone secretion in female ruminants. In: Opioids in farm animals, Ed. N.Parvizi, Landwirtschaftsverlag GmbH, 4400 Münster-Hiltrup, Germany. pp.11-14

Shaar, C.J., Clemens, J.A. (1980). The effect of opiate agonists on growth hormone and prolactin release in rats. Fed. Proc., 39:2539-2543.

Shaw, H.J, Foxcroft, G.R. (1985). Relationship between LH, FSH, and prolactin secretion and reproductive activity in the weaned sow. J. Reprod. Fertil., 75:17-28.

Smith, C.A, Almond, G.W, Dial, G.D. (1992). Changes in luteinizing hormone concentrations after abortion, parturition, and weaning in primiparous sows. Anim. Reprod. Sci., 27:169-182.

Stevenson, J.S., Britt, J.H. (1981). Interval from weaning to oestrus in the sow and performance of pigs after alteration of litter size during late lactation. J. Anim. Sci., 53:1, 177-181.(suppl. 1), 393.

Tokach, M.D., Pettigrew, J.E., Dial, G.D., Wheaton, J.E., Crooker, B.A., Johnston, L.J. (1992). Characterization of LH secretion in the primiparous lactating sow:relationship to blood metabolites and return to estrus interval. J. Anim. Sci. 70:2195-2201.

Weisner, J.B., Koening, J.I., Krulich, L., Moss, R.L. (1985). Possible delta receptor mediation of the effect of beta-endorphin in LH release, but not on prolactin release in the ovariectomized rat. Endocrinology, 116:475.

Zukin, R.S, Zukin, S.R. (1981). Multiple opiate receptors: Emerging concepts. Life Sci. 29:2681-2693.

# CHAPTER 5

# EXPERIMENT 4: RELATIONSHIP BETWEEN GONADOTROPIN SECRETION DURING LACTATION AND ENDOCRINE CHANGES AND FOLLICULAR DEVELOPMENT AFTER WEANING IN THE SOW.

## 5.1 INTRODUCTION

The development of a method for evaluating the potential fertility of sows by determining their gonadotropin status would be of great practical importance. Research on FSH currently suggests a positive correlation between the peripheral plasma concentration of FSH in the female lamb and genetic merit for litter size (Ricordeau et al., 1984). Since endogenous LH release is pulsatile and accurate characterization of LH secretion requires frequent sampling, it is a poor candidate as an indirect indicator of fertility. However LH release after GnRH injection is more repeatable. Studies of the prolific Finnish Landrace ewe and its crosses, in comparison with lambs from less prolific breeds,suggested a correlation between LH responses to GnRH and the index of prolificacy (Land and Carr, 1979). While the later study of Hanrahan et al. (1981) did not support this conclusion, Haley et al. (1989) suggest that there is a different response between low- and high-line ewes to GnRH stimulation at puberty or at the beginning of the breeding season.

One injection of GnRH is able to significantly increase LH secretion during lactation in the pig (Bevers et al.,1981; Rojanasthien et al.,1987) and the gonadotropin responses to GnRH injection have been used to characterize reproductive status in prepubertal gilts (Deligeorgis et al., 1985). The injection of opioid antagonists (i.e. Naloxone) is also able to increase LH secretion during lactation (see Barb et al.,1991; De Rensis et al.,1993a,b; Britt et al., 1993).

The aim of this study was determine if there is a correlation between endogenous LH secretion and LH secretion after GnRH and naloxone injection during lactation, and the increase in gonadotropin and follicular development after weaning.

## 5.2 MATERIALS AND METHODS

### 5.2.1 Animals and blood collection

A total of nine multiparous Camborough sows (PIC, Acme, AB., Canada) from the University of Alberta herd were used. Sows were housed in conventional farrowing crates and water was provided ad libitum throughout lactation. A standard lactating sow ration was provided on the basis of body weight and lactation requirements for each sow. The sows were fed twice daily at 9.00am and 4.00pm. The daily food intake and the body weight loss during lactation in each sow were determined. The average weight of the sows one day after farrowing was 175±1.2Kg and the average litter size during the experiment was 9.7±0.7 piglets.

All animals were cannulated non surgically using ear vein catheters 22 to 24h after the end of farrowing. Blood samples (2.5 ml) were taken from all sows at 15 min intervals for 6h at day 2 post-partum. The catheters were removed as soon as possible after this initial period of sampling. Seven days before weaning at day 25 of lactation all the sows were provided surgically with indwelling jugular catheters under general anaesthesia. Further 15 min samples were then obtained for a 12h period on day 25 of lactation and for 6h starting six hours after weaning at day 26 of lactation. Four hours after the beginning of sampling at day 25 of lactation all sows were injected with 2mg/kg i.v. of naloxone hydrochloride and 5h later with GnRH (100 ug/sow).

### 5.2.2 In vitro study

Follicular development was studied in the left ovary of all sows the day after weaning; the other ovary was used for mRNA extraction for analyses not reported in this paper. Follicular development was assessed by measuring the volume of follicular fluid recovered from the ten largest follicles.

### 5.2.3 Hormone assays

Plasma LH was quantified in all samples. Plasma LH concentrations were determined using the double antibody RIA described by De Rensis et al. (1993b). The inter- and intra-assay coefficients of variation were 4.1% and 12.7%, respectively. The sensitivity of the assay, defined as 90% of total binding, was 0.13ng/ml.

### 5.2.4 Statistical analysis

The jugular cannulae of two gilts failed to remain patent throughout the bleeding period at day 25 and data from these animal were not included in the analyses.

Hormone data from 6h sampling at day 2 post-partum and after weaning were derived as means of a single period of 6h (periods 1 and 6, respectively). Data from the 12h sampling period at day 25 of lactation were characterized as means representing a 4h period before naloxone injections (period 2), a 2h period after naloxone injections (period 3), a 3h period before GnRH injections (period 4) and a 3h period after GnRH injections (period 5). Comparison of means for periods 1, 2 and 6 were analyzed separately using a repeated measures analysis of variance within the PROC GLM procedure of the SAS statistical package (SAS,1985). In the presence of significant period effects specific comparisons between these means was made using Student-Newman-Keuls multiple range test. Treatment effects (naloxone or GnRH) during sampling on day 25 of lactation were analyzed using a priori comparisons between means for periods 2 and 4, and periods 2 and 3, by the same statistical procedures. Having established a significant difference between period 2 and 4, the response to GnRH challenge (period 5) was tested using repeat measures analysis between period 2 and 5, fitting period 4 as a covariate.

Differences in follicular volume were examined by PROC ANOVA with sow as the main effect. Regression analysis was used to determine possible relationships between follicular characteristics and mean plasma LH concentrations and among different LH means.

## 5.3 RESULTS

The individual patterns of LH secretion in four sows are shown in Fig. 5.1 and 5.2. Mean plasma LH concentrations are showed in Fig. 5.3. There was an effect of time (sampling period; $p<0.001$) on mean plasma LH, with a significant ($p<0.05$) increase in

LH the day after weaning (period 6) compared to mean LH concentrations during lactation (periods 1 and 2). Naloxone ($p<0.05$) and GnRH treatment significantly ($p<0.05$) increased mean LH concentrations. There were no relationships between the response of LH to naloxone and to GnRH treatments. However a positive relationship ($r=0.58$, $p<0.01$) between mean plasma LH after GnRH (period 5) and after weaning (period 6) was established. Finally there were no relationships between mean plasma LH at day 2 (period 1) and after weaning (period 6). There was a significant ($p<0.001$) difference between sows in follicular development (Fig.5.3) but there were no correlations between follicular fluid volume and mean LH secretion in periods 5 and 6.

## 5.4 DISCUSSION

It has been reported (Shaw and Foxcroft 1985) that mean LH concentrations during late lactation, but not after weaning, are greater for sows showing estrus within four days of weaning, suggesting that the endocrinology of the sow during lactation can influence the resumption of estrus after weaning. Furthermore data of Tokach et al. (1992) indicate that alterations in LH secretion that lead to a delayed return to estrus postweaning in the sow may occur as early as day 14 of lactation. These differences however could be due to differences in nutrient intake during lactation, as has been shown in the cow (Short et al.,1990; Rusche et al., 1993) and sow (King et al., 1989).

In the present study, in contrast to the results from Shaw and Foxcroft (1985) and Tockach et al. (1992), no correlation between LH concentrations during lactation and the day after weaning was established. This could be due to the lower number of animals utilized in this study compared to the studies of Shaw and Foxcroft (1985) and Tokach et al. (1992). However, our data indicate that the endocrinology of sows in the early post partum period cannot be utilized as a predictor of LH activity at weaning.

Haley et al. (1989) classified two lines of lambs as high or low responders on the basis of the parental response to GnRH. They observed a more rapid response of LH to GnRH stimulation in their progeny and this was positively correlated with body weight and mean testis diameter, suggesting therefore that selection on the basis of the LH response on GnRH is possible. In the present experiment there was a positive correlation between LH means after GnRH treatment and after weaning. These observations indicate that the utilization of GnRH injections before weaning could be a predictor of the reproductive performance of the sows after weaning.

The opioids are involved in the mechanisms mediating the inhibitory effect of suckling stimulus on LH secretion (see Barb et al., 1991; De Rensis et al., 1993a; Britt et al., 1993). In the present experiment naloxone increased LH concentrations, but a more substantial increase of LH was observed after GnRH administration. These results confirm and extend the observations of Barb et al. (1986) and indicates that in pigs, naloxone alone is not able to totally disinhibit the block of LH secretion due to the suckling stimulus. Therefore, these data suggest that there are some opioid receptors not affected by naloxone or other factors involved in mediating the inhibitory effect of the suckling stimulus on LH secretion during lactation. However, it has to be considered that the differences in mean LH response between naloxone and GnRH could be related to the possibility that the dose of GnRH used was slightly supra physiological. The absence of a correlation between plasma LH after GnRH injection during lactation and follicular development after weaning indicate that the ability to respond to GnRH stimulation is not the only factor determining an earlier resumption of follicular development after weaning.

**Fig. 5.1** Individual plasma LH profiles in two sows sampled for 6, 12 and 6h on day 2 and 26 post-partum and the day after weaning, respectively. Four hours after the beginning of sampling at day 25 of lactation the sows were injected with 2mg/kg i.v. of naloxone and 5h later with GnRH (100 ug/sow).

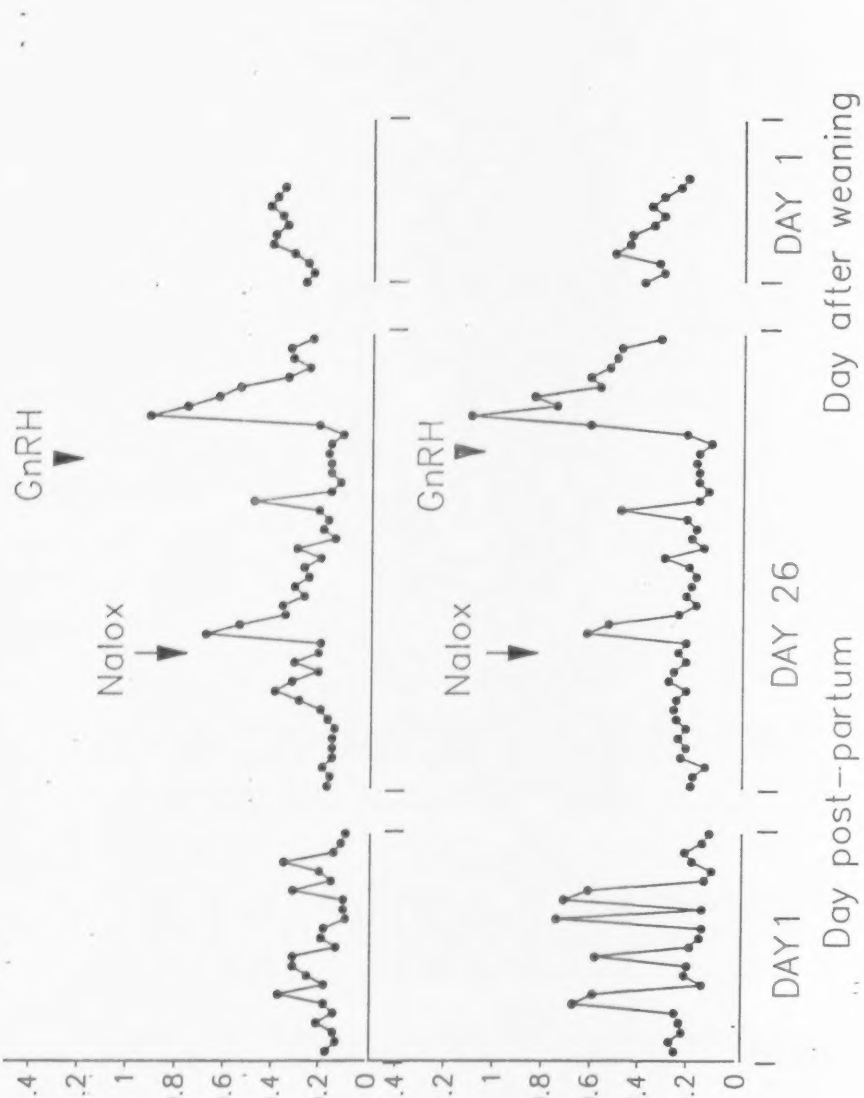
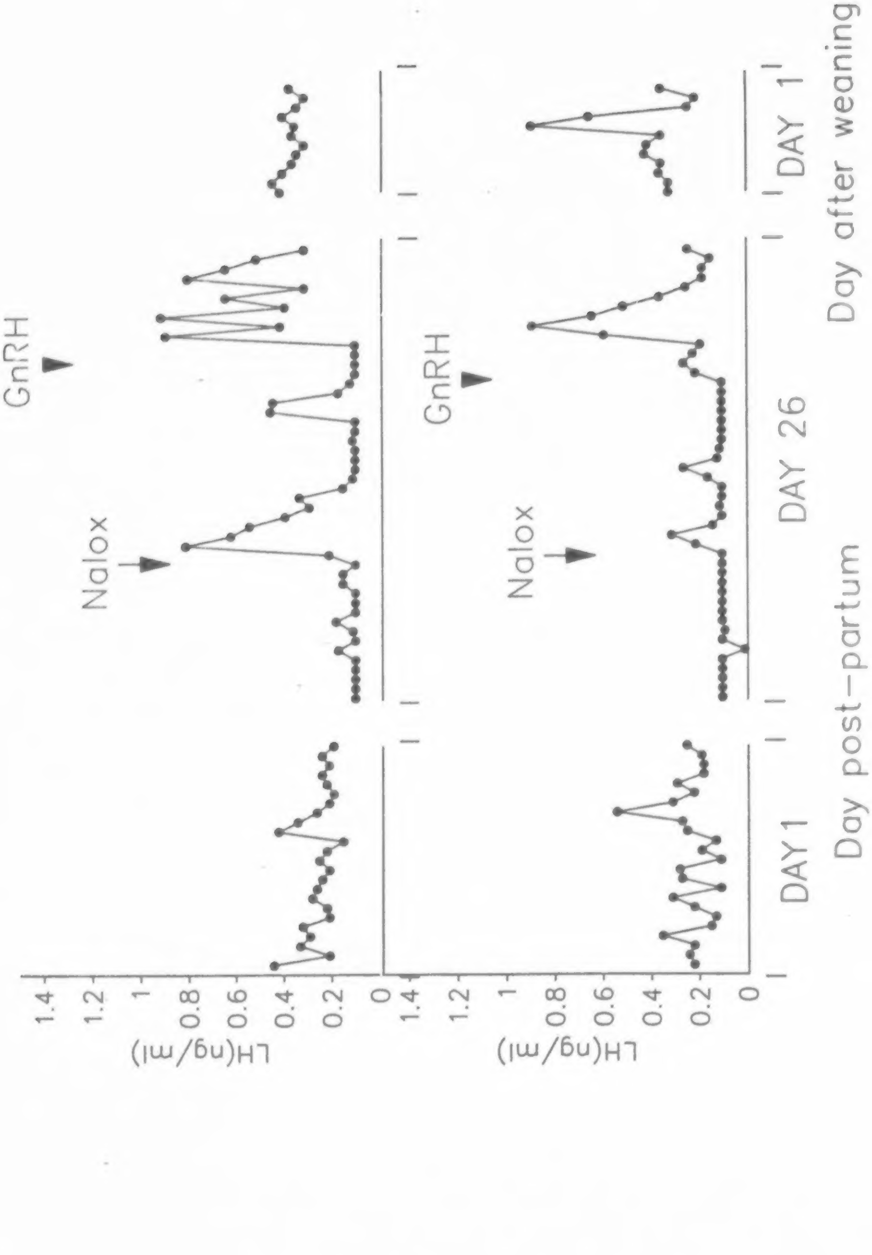

GnRH
Nalox
GnRH
Nalox
LH(ng/ml)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
LH(ng/ml)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
DAY1
DAY 26
DAY 1
Day post-partum
Day after weaning

**Fig. 5.2** Individual plasma LH profiles in two sows sampled for 6, 12 and 6h on day 2 and 26 post-partum and the day after weaning, respectively. Four hours after the beginning of sampling at day 25 of lactation the sows were injected with 2mg/kg i.v. of naloxone and 5h later with GnRH (100 ug/sow).



LH(ng/ml)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
LH(ng/ml)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Nalox
GnRH
Nalox
GnRH
DAY 1
DAY 26
DAY 1
Day post-partum
Day after weaning

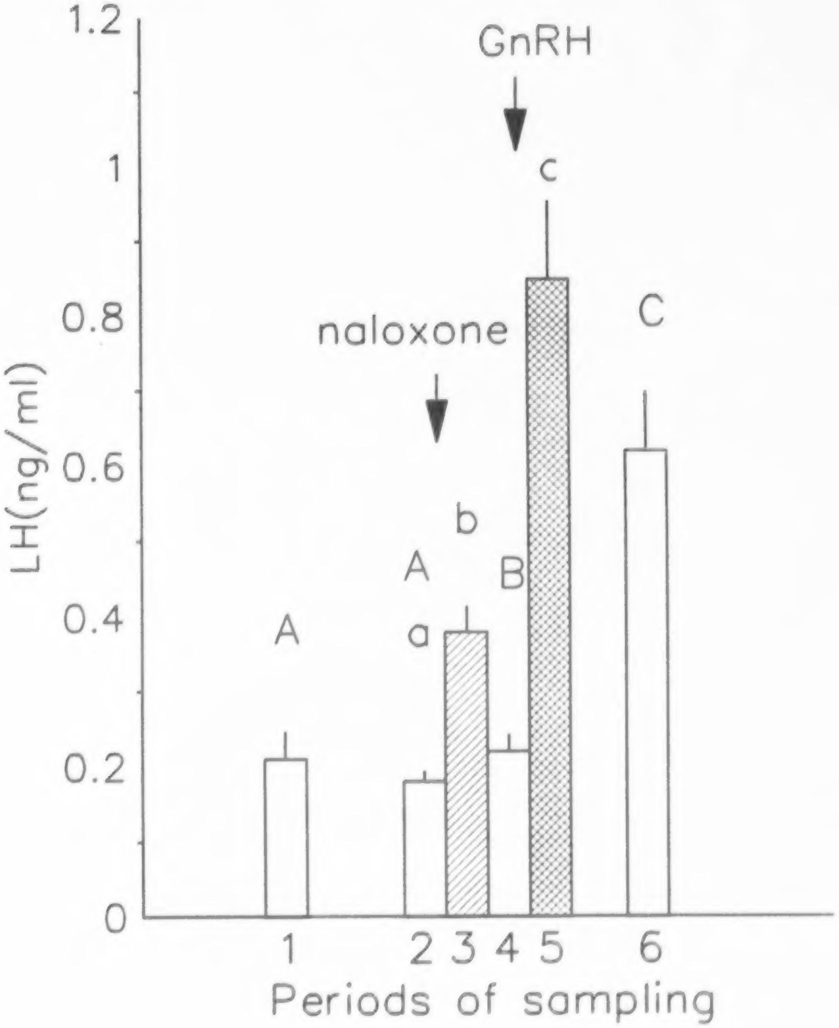
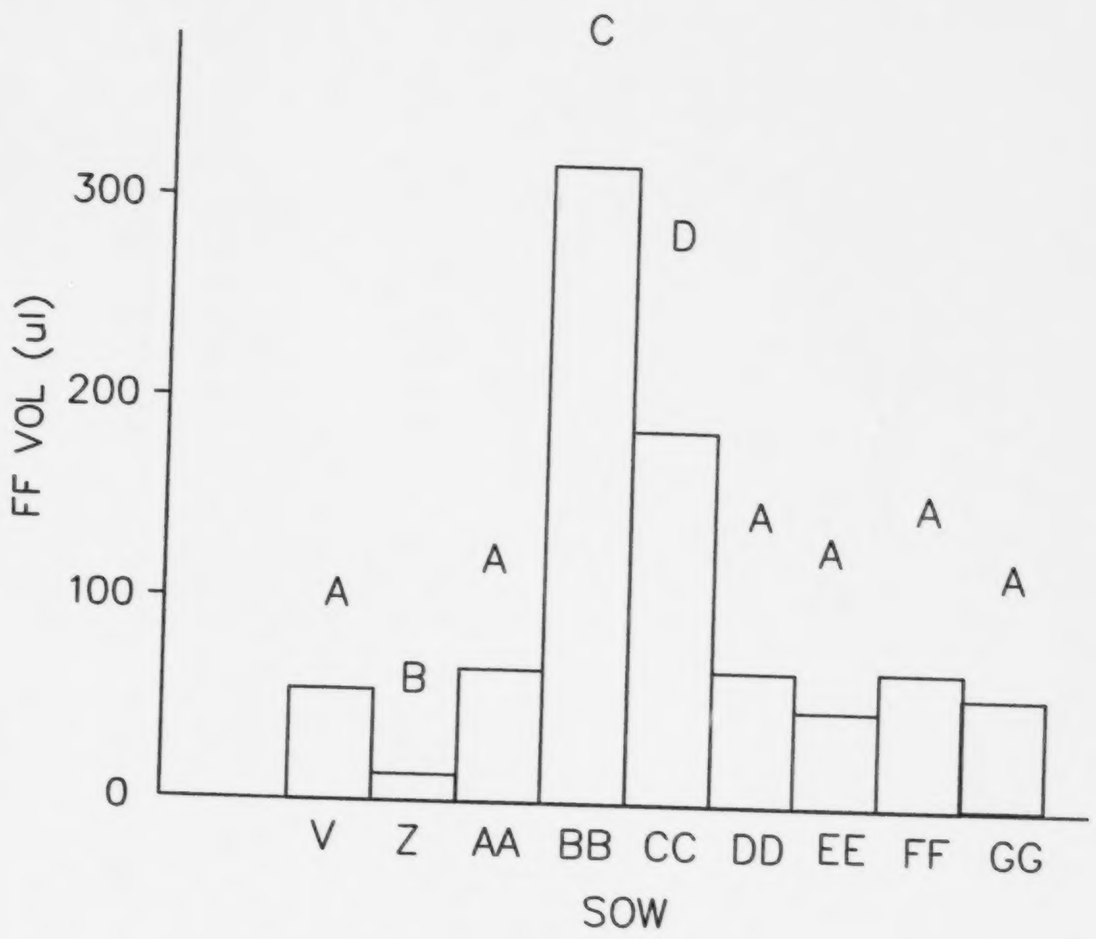


**Fig. 5.3** Mean (+SEM) plasma LH during the six periods of sampling two days after farrowing (Period 1 = 6h), at day 25 of lactation (Periods 2, 3, 4 and 5 of 4h, 2h, 3h and 3h, respectively) and the day after weaning (Period 6 = 6h). Four hours after the beginning of sampling at day 25 of lactation the sows were injected with 2mg/kg i.v. of naloxone and 5h later with GnRH (100 ug/sow). Means with different superscripts differ ($p<0.05$).

**Fig. 5.4** Mean follicular volume (ul) for the ten largest follicles on the left ovary for each sow in the experiment. Means with different superscripts differ ($p<0.001$).


C
D
A
B
A
A
A
A
A
300
200
100
0
FF VOL (ul)
V
Z
AA
BB
CC
DD
EE
FF
GG
SOW

## 5.5 BIBLIOGRAPHY

Barb, C.R., Kraeling, R.R., Rampacek, G.B., Whisnant, C.S. (1986). Opioid inhibition of luteinizing hormone secretion in the post-partum lactating sow. Biol. Reprod., 35:368-371.

Barb, C.R., Kraeling, R.R., Rampacek, G.B. (1991). Opioid modulation of gonadotropin and prolactin secretion in domestic farm animals. Dom. Anim. Endocrinol., 91:15-27.

Bevers, M.M., Willemse, M.H., Kruip, T.A.M., Van de Wiel D.F.M. (1981). Prolactin levels and the LH response to synthetic LHRH in lactating sow. Anim. Reprod. Sci., 4:155-163.

Britt, J.H., Armstrong, J.D., Moore, K.L., Sesti, L.A.C. (1993). Involvement of opioids in regulation of LH secretion during lactational- or nutritional-induced anestrus in pig and cattle. In: Opioids in farm animals, Ed. N.Parvizi, Landwirtschaftsverlag GmbH, 4400 Münster-Hiltrup, Germany. pp. 34-54.

Deligeorgis, S.G., English, P.R., Lodge, G.A. (1985). Interrelationships between growth, gonadotrophin secretion and sexual maturation in gilts reared in different litter sizes. Anim. Prod., 41:393-401.

De Rensis, F., Foxcroft, G.R. (1993a). Endogenous opioid modulation of gonadotropin and prolactin secretion in the post-partum sow. In: Opioids in farm animals, Ed. N.Parvizi, Landwirtschaftsverlag GmbH, 4400 Münster-Hiltrup, Germany. pp. 15-33.

De Rensis, F., Hunter, M.G., Foxcroft, G.R. (1993b). Suckling-induced inhibition of LH secretion and follicular development in the early post-partum sow. Biol. Reprod, 48:964-969.

Haley, C.S., Lee, G.J., Fordyce,M., Baxter, G., Land, R.B., Webb, R. (1989). Study of LH response to GnRH in the young female as a criterion of genetic merit for female reproduction in the sheep. J. Reprod. Fertil. 86:119-133.

Hanrahan, J.P., Quirke, G.F., Gosling, G.P. (1981). Effect of lamb age, breed and sex on plasma LH after administration of GnRH. J. Reprod. Fertil., 61:281-288.

Hanrahan, J.P. (1982) Selection for increased ovulation rate, litter size, and embryo survival. Proc. 2nd Wld Congr. on Genetics Applied to Livestock production, Editorial Garsi, Madrid, 5, 294-309.

King, R.H., Martin, G.B. (1989). Relationship between protein intake during lactation, LH levels and oestrus activity in first litter sows. Anim. Reprod. Sci., 19:283-288.

Land, R.B., Carr, W.R. (1979) Reproduction in domestic mammals, In:Genetic Variation in Hormone Systems, pp. 89-112. Ed. J.G.M. Shire, CRC Press, London.

Mullan, B.P., Close, W.H. (1989) The partition of nutrients during lactation and its relationship to reproductive performance. In: Manipulating Pig Production II. Aust. Pig Sci. Assoc., Werribee, Australia. Eds. J.L. Barnett and D.P. Hennessey, p 302.

Ricordeu, G., (1982) Selection for reduced seasonality and post-partum anoestrus. Proc. 2nd Wld Congr. on Genetics Applied to Livestock production, Editorial Garsi, Madrid, 5, pp.338-347.

Rojanasthien, S.A.M., Lundeheim, N., Einarsson, S. (1987). Luteinizing hormone response to different doses of synthetic gonadotrophin releasing hormone during early and late lactation in primiparous sows. Anim. Reprod. Sci., 13:299-307.

Rusche, W.C., Cochran, R.C., Corah, L.R., Stevenson J.J., Harmon D.L., Brandt, R.T., Minton, J.E. (1993). Influence of source and amount of dietary protein on performance, blood metabolites, and reproductive function of primiparous beef cows. J. Anim. Sci., 71:557-563.

Shaw, H.J., Foxcroft, G.R. (1985). Relationship between LH, FSH, and prolactin secretion and reproductive activity in the weaned sow. J. Reprod. Fertil., 75:17-28.

Short, R.E., Bellows, R.A., Staigmiller, R.B., Berardinelli, J.C., Custer, E.E. (1990) Physiological mechanisms controlling anestrus and infertility in postpartum beef cattle. J. Anim. Sci., 68:799.

Tokach, M.D., Pettigrew, J.E., Dial, G.D., Wheaton, J.E., Crooker, B.A., Johnston, L.J. (1992). Characterization of LH secretion in the primiparous lactating sow:relationship to blood metabolites and return to estrus interval. J. Anim. Sci. 70:2195-2201.

## CHAPTER 6

## EXPERIMENT 5: CHRONIC ADMINISTRATION OF A LONG ACTING DOPAMINE AGONIST (CABERGOLINE) BLOCKS THE INHIBITORY EFFECT OF OPIOIDS ON LH SECRETION DURING LACTATION IN THE SOW.

### 6.1 INTRODUCTION

The post-partum period in cows, sheep and pigs is characterized by anestrus and a lack of ovarian activity, primarily due to an inhibition of GnRH and thus of gonadotropin secretion. Although it is known that GnRH is secreted in specialized neurons in the hypothalamus, little is known about their immediate control. Evidence derived from laboratory species indicates that at least noradrenaline, dopamine, serotonin and opioids are involved in the control of the release of GnRH (see Weiner et al., 1988). There is limited information on such control pathways in farm animals, particularly during the post-partum period. On an anatomical basis there is evidence for juxtaposition of catecholaminergic and GnRH neurones (Lehman et al., 1988) and in many situations elevated plasma PRL concentrations are associated with reduced gonadotropin secretion (for review see McNeilly, 1987).

By the use of dopamine infusion or dopamine agonists and antagonists it has been demonstrated in different species that dopamine has either a stimulatory (Sneider and McCann 1970; Jackson, 1977; Rotstzejin et al., 1977; Rasmussen et al., 1986) or an inhibitory effect on LH release (Uemura and Kobayashi, 1971; Miyachi et al., 1973; Drouva and Gallo, 1976; Beck et al., 1977; Deaver and Dailey, 1983; Ramirez et al., 1984; Deaver et al., 1987; Kuljis et al., 1989). Studies in humans consistently indicate that dopamine has an inhibitory effect on LH secretion (Leebaw et al., 1978; Andersen et al., 1987). In the rat, it has been suggested that one mechanism by which PRL can interfere with gonadotropin activity is by causing an increase in dopamine turnover in neurons of the median eminence and as a consequence, enhanced dopaminergic tone may influence gonadotropin secretion by blocking release of GnRH (Evans et al., 1980). However, the results of Demarest et al. (1983) seems to exclude this mechanism in lactating animals. However there are studies (Muralidhar et al., 1977; Marchetti et al., 1982) indicating that the hypothalamic-hypophysial axis becomes more sensitive to the suppressive effects of prolactin with time from farrowing. The dopaminergic system seems to be involved in the regulation of gonadotropin secretion. Treatment with the dopamine agonist bromocriptine restored ovulatory function in women with hyperprolactinaemia (Besser et al., 1972; Del Pozo et al., 1974; Thorner et al., 1975). There is evidence supporting a role of endogenous opioids in the control of GnRH, PRL and LH secretion during lactation in pigs, sheep and cattle (for review see Peters and Lamming 1991; De Rensis et al., 1993a). In the sow the opioids have been shown to be important mediators of suckling effects on LH and PRL secretion (Mattioli et al., 1986; Barb et al., 1986; Armstrong et al., 1988a,b; De Rensis et al., 1993c). The principal mechanism(s) by which opioids control gonadotropin secretion during lactation is not completely understood. The mechanism(s) by which opioids control prolactin secretion during lactation it also not well understood. An inhibitory effect on tuberoinfundibular dopaminergic neurons and an increase in secretion of prolactin releasing factors have been suggested (for review see Ben-Jonhathan et al., 1988).

There are data indicating that hyperprolactinaemia interferes with the opioidergic regulation of LH. In ovariectomized rats that were rendered hyperprolactinaemic by ectopic pituitary grafts, administration of naloxone resulted in a greater increase in plasma LH than that observed in ungrafted controls (Sarkar et al., 1985) and hypothalamic levels of beta-endorphin and metenkephalin are increased in hyperprolactinemic animals (Panerai et al. 1980). Taken together these observations suggest that opiates exert a stronger tonic inhibition on GnRH secretion in the presence of elevated prolactin concentrations. With regard to the pig, Mattioli and Seren (1985) found no differences in LH secretion after bromocriptine treatment, whilst Kraeling et al. (1982) observed that treatment with bromocriptine was associated with significantly lower serum LH concentrations in treated animals compared to controls. Finally, Bevers et al. (1983) found that the reduction of plasma prolactin concentrations by bromocriptine significantly increased mean LH concentrations. These discrepancies could be due to differences in the dose, the length of treatments and the period of lactation at which the treatment was given.

Recently a new long acting prolactin inhibitory agent (Cabergoline) has been synthesized which is effective in modifying PRL secretion during lactation in the rat, rabbit, dog, cat (Di Salle et al., 1983) and pig (Maffeo et al., 1989). The primary objective of this experiment was therefore to study the effect of chronic treatment with Cabergoline on the regulation of gonadotropin and prolactin secretion during lactation in the sow. Because of the potential interactions between the dopaminergic and opioidergic systems reviewed above, the effects of opioid regulation on the response to the dopamine agonist was also investigated, by the administration of morphine or naloxone in Cabergoline treated sows. The second part of the experiment extended these results at the most critical response period at day 26 of lactation.

## 6.2 MATERIALS AND METHODS

### 6.2.1 Animals and blood collection

A total of 27 multiparous crossbred sows were used. Sows were housed in conventional farrowing crates and water and a commercial lactating sow ration were provided ad libitum throughout lactation. The average weight of sows one day after farrowing was 168.0±6.3kg and the average litter size during the experiment was 9.1±0.7 piglets. Sows were provided non-surgically with indwelling jugular catheters two hours before the start of each sampling period using jugular venipuncture during nose-snare restraint. This approach avoided damage to chronically placed cannulae by piglets older then 12 days. Blood samples (2.5 ml) were taken from all sows at 15 min intervals during all sampling periods.

In part I, sixteen sows were randomly allocated to one of four treatments (n=4) and were sampled every 15 min for 6h periods on days 12, 19 and 26 of lactation. To provide possible evidence for the development of estrogenic follicles in response to treatment, single 10 ml samples were taken during each sampling window in all animals for evaluation of plasma estradiol-17beta. Control sows (CON) received no treatment; Cabergoline (CAB) sows received 250mcg of Cabergoline, a new ergot derivate (FCE

21336 Farmitalia, Milan, Italy) orally twice a day from day 11 to day 26 of lactation as a top dressing on freshly provided food; Cabergoline and Naloxone (CAB+NAL) sows were treated with Cabergoline as in group CAB and 3h after the start of each sampling period sows also received an i.v. injection of 2mg/kg naloxone hydrochloride (Sigma Chemical Co., St. Louis, MO) followed by multiple injections every 30 min of a total of 1.1 mg\kg\h of naloxone for 3h according to the dose regimen of Amstrong et al. (1988). Cabergoline and Morphine (CAB+MORPH) sows were treated with Cabergoline as in group CAB and 3h after the start of each sampling period sows also received a single injection of 0.5 mg/kg of Morphine sulfate (SALARS, Como, Italy).

In part II of the experiment, a further 11 sows were allocated to either Control (n=4), Cabergoline (n=4) or cabergoline and morphine (n=3) treatments as in part one. However, in this part of the study the effects of treatment were only confirmed in a single period of sampling for 6h every 15 min on day 26 of lactation.

### 6.2.2 Hormone assays

Plasma LH was quantified in all samples using the double antibody RIA described by De Rensis et al. (1993). The inter- and intra-assay coefficients of variation were 14.7 and 9.82 %, respectively. The sensitivity of the assay, defined as 90% of total binding, was 0.13ng/ml.

PRL was quantified in 30 min samples obtained in part one of the experiment by the method described by Shaw and Foxcroft (1985) with minor modifications. The intra-and inter-assay coefficients of variation were 9.9% and 17.1%, respectively. The overall sensitivity of the assay, defined as 85% of total binding, was 1.9ng/ml.

Plasma concentrations of estradiol-17beta were analyzed in one assay with an intraassay c.v. of 14.1% The sensitivity of the assay was 2.8 pg/ml at 90% binding.

### 6.2.3 Statistical analysis

Subjective assessment of the plasma LH profiles obtained in this study indicated that the adoption of computerized pulse analysis programs was inappropriate (for details see Foxcroft et al., 1988). The 6h hormone profiles for each of the three sampling periods (days) were therefore analysed as means of two 3h periods. In the absence of any replicate effect, the results of experiment II were pooled with appropriate data from experiment I at day 26.

#### Part 1

The repeated measures analysis of variance within the PROC GLM procedure of the SAS statistical package (SAS, 1985) was utilized to test for cabergoline effects using a model fitting group (CONT or CAB) as a main effect, with day of sampling (day 12, 19 and 26) as a repeated measurement. When significant interactions were established, appropriate least square means were compared by Student-Newman-Keuls multiple range test. To evaluate linear, quadratic and cubic trends for time, polynomial contrasts were used.

#### Part 2.

The repeated measures analysis of variance was used to test for the effect of naloxone in CAB+NAL sows or of morphine in CAB+MORPH within days and the effect of days. When significant interactions were established, appropriate least square means were compared by Student' t test.

## 6.3 RESULTS

The mean piglet weight at day 12, 19 and 26 for Control sows were 3.41, 4.81 and 6.9 kg., and for Cabergoline sows 3.27, 4.21 and 7.1 kg., respectively. There were no differences in weight between the piglets of the Control sows and the piglets of the Cabergoline treated sows. These data are in agreement with a previous study (Maffeo et al., 1989), in which the administration of 250 mg of cabergoline to lactating sows did not significantly modify litter weight and hence milk production.

### Plasma LH

The individual patterns of LH secretion in two sows of each group are shown in Fig. 6.1 and 6.2. The overall treatment means in CONT and CAB groups for mean plasma LH are shown in Fig. 6.3a and for CAB+NAL and CAB+MORPH groups in Fig. 6.3b. The statistical analyses of the data established a significant ($p<0.001$) difference in mean plasma LH concentrations between Cabergoline and control sows at day 26 of lactation (Fig. 6.3b). The multiple naloxone injections significantly increased ($p<0.05$) mean plasma LH at day 12 ($p<0.1$) and 19 ($p<0.05$) of lactation, but no effect of naloxone was observed at day 26 of lactation. Administration of morphine decreased ($p<0.05$) mean plasma LH at day 26 of lactation only.

### PRL

The individual patterns of PRL secretion in two sows of each group are shown in Fig. 6.5 and 6.6 and the overall treatment means in Fig. 6.7. Cabergoline treatment significantly ($p<0.05$) decreased mean prolactin plasma levels. There were no effects of naloxone or morphine on prolactin secretion at any stage of the experiment. In suckled sows plasma PRL was already elevated at the first period of sampling and there was no change in plasma PRL concentration over the subsequent periods of sampling. In the Cabergoline treated sows plasma PRL was already low at the beginning of sampling and did not change with time.

### Plasma Estradiol

Plasma estrogen concentrations were below 5 pg/ml for all sows during all periods of sampling.

## 6.4 DISCUSSION

Mean plasma LH concentrations observed in this study in control sows were similar to our previous observations (De Rensis et al., 1993c) and data from other studies (Barb et al., 1986; Mattioli et al., 1986; Tokach et al., 1992). In this study a stimulatory effect of Cabergoline on LH secretion was observed at day 26 but not at day 12 and 19 of lactation. Therefore these data suggest that in the sow, chronic Cabergoline administration can disinhibit the inhibitory effect of the suckling stimulus on LH secretion. These results are different from observations from studies of dopamine agonist effects in humans (Martin et al., 1981; Pontiroli et al., 1985) and in the sow (Bevers et al., 1983; Kraeling et al., 1982). However, these differences may be due to the type of treatment. For instance, Krealing et al. (1982) injected 120mg of bromocriptine subcutaneously during their experiment and compared the plasma LH levels immediately before and after the injections of bromocriptine. In contrast, in our study the first period of sampling occurred when the sows had already been treated with Cabergoline for 24 hours and we may therefore have missed any immediate response to initial treatment. Other studies in which the administration of bromocriptine was not able to modify LH secretion have also been reported in humans (Tolis et al., 1975; Evans et al., 1980) and in sows (Mattioli et al., 1985). LH secretion was eventually increased by treatment with Cabergoline at day 26 of lactation in the present study. This suggests that either sows became more responsive to dopamine agonist treatment as lactation progressed, or that chronic rather than acute oral administration of Cabergoline is needed to produce an effect. Support for the latter suggestion comes from the observation that bromocriptine administration to hyperprolactinemic patients did not induce any changes in basal gonadotropin secretion during the first days of treatment (Djursingt et al., 1981) but a normal rate of LH pulsatility was restored with chronic treatment (Moult et al., 1982).

There are few studies in the literature regarding the effects of treatment with dopamine agonists on follicular development. In a recent study in post-partum women using bromocriptine and a new non-ergot dopamine agonist (CV205-502). Kremer et al. (1991) reported that there were no differences in LH secretion but an anticipated return of pituitary and ovarian activity in bromocriptine and CV205-502 treated, compared to non-treated bottle-feeding woman. However, the absence of differences in plasma LH between the two groups could be related to the frequency of the blood samples (one per day) used in the experiment, which would not allow a precise description of LH secretion. However, these data suggest that the administration of dopamine agonists can stimulate ovarian activity during lactation.

The site(s) at which bromocriptine acted to disinhibit LH release in post-partum sows cannot be determined from the present experiment. With regard to the distribution of dopamine receptors, there are studies indicating that D2 receptors are found in the pituitary, whereas the dopamine receptors of the median eminence are primarily of the D1 type (Leibowitz et al., 1982). In a recent study Martinez-de-la-Escalera et al. (1992) and Findell et al. (1993) report that there is a direct stimulatory effect of DA on GnRH release via dopamine D1-receptors but not dopamine D2-receptors. Schoors et al. (1991) and Boesgaard et al. (1991) also identified D1 receptors in pituitary cells and the administration of fenoldopan (a specific dopamine D1 receptor agonist) during the follicular phase in woman increased the LH and FSH responses to GnRH administration. However there are studies reporting that Cabergoline can cross the blood-brain barrier and can interact with central dopamine receptors (Jori et al., 1987; Benedetti et al., 1990). Multiple opioid receptor types have been implicated in suppression of LH secretion. Morphine and naloxone do not have identical affinities for all opioid receptors types. Both bind with high affinity to mu1- and mu2- receptors (Lord et al., 1977; Casy et al., 1986; Pasternak et al., 1986a,b; Kazmi et al., 1987) but naloxone also binds to delta-receptors (Khatchaturian et al., 1985). In the literature there are data that suggest that an interaction may exist between the dopaminergic and opioidergic systems in the control of LH secretion (Van Vugt et al., 1981; Laedem et al., 1985; Petersen et al., 1986). Finally

Drouva et al. (1980, 1981) observed that the opioids may inhibit dopamine or potassium evoked release of LHRH from LHRH terminals. Also, direct effects of beta-endorphin on dopamine secretion have been demonstrated (for review see Haynes et al., 1989; Ben Jonathan et al., 1988).

However, in some studies an effect of dopamine on opioid secretion has also been identified. There are in fact data showing that in humans tonic dopamine inhibition of beta-endorphin secretion exists (Genazzani et al., 1984). Stimulatory effects of DA antagonists on beta-endorphin secretion have been observed in rats (Giraud et al., 1980; Farah et al., 1983) and dogs (Sharp et al., 1982). In the ram, dopamine inhibited beta-endorphin and PRL secretion under either long or short days (Ssewannyana et al., 1990). Furthermore, Autelitano et al. (1987) report that dopamine receptor agonists (bromocriptine, lysuride) do not change plasma beta-endorphin when acutely administered but decrease beta-endorphin after chronic treatment. Finally, in vitro studies demonstrated that the release of beta-endorphin from the mediobasal hypothalamus is under the inhibitory control of dopaminergic neurons (Vermes et al., 1984). Although there was considerable variability between animals, naloxone treatment in lactating sows at day 15 (Mattioli et al., 1986), day 21 (Armstrong et al., 1988b) and at day 22 (Barb et al., 1986) have a stimulatory effect on LH secretion. Armstrong et al. (1988a) report that acute administration of morphine prevents the increase in LH secretion associated with transient weaning and that chronic administration delayed onset of estrus after weaning, presumably through suppression of LH secretion. In the human it has been shown that bromocriptine has a positive effect on the LH response to opioid antagonists (Melis et al., 1988). However in the present work, the absence of a naloxone effect and the presence of an inhibitory effect of morphine in CAB+NAL and CAB+MORPH groups, respectively, at day 26 of lactation may be due to a disinhibition of the opioidergic effect on GnRH and thus LH secretion by Cabergoline treatment. In conclusion, these observations suggest that an interaction between dopaminergic and opioidergic systems during lactation in the sow, in controlling LH secretions is possible.

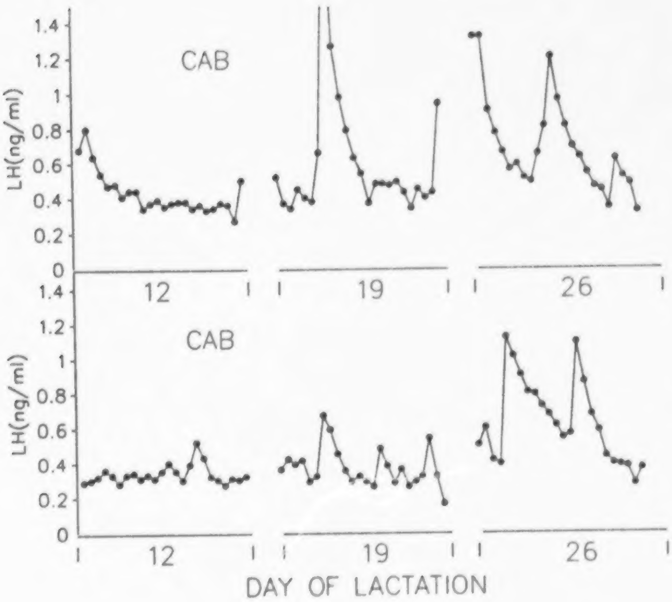
There are data suggesting a direct effect of PRL on LH secretion. When lactating rats are ovariectomized in midlactation (day 10-15), suckling and/or prolactin reduced to 50%, but did not completely prevent, an increase in gonadotropins (Smith, 1981). Some studies suggest that prolactin alone in the absence of ovarian steroids can augment the inhibitory effect of the suckling stimulus and this effect is apparent in later stages of lactation when the suckling stimulus is declining (Smith, 1978; Hansen, 1983). In the present study, unchanged plasma LH concentrations in the presence of low plasma prolactin in treated sows, for at least eight days after the beginning of the treatment with Cabergoline, suggests that prolactin per se does not have a direct effect on LH secretion during lactation. These observations therefore further support studies that suggest that prolactin per se may play only a minor role on control of gonadotropin release post-partum (McNeilly, 1988).

Plasma estradiol concentrations were very low throughout the experiment and did not change with time after farrowing, either in the presence or absence of Cabergoline treatment, indicating a lack of marked follicular development. However, because a rise in plasma estradiol would only be expected at the time that estrogenic follicles were present this is a relatively insensitive estimate of early follicular development. Without direct observation of follicle status at the end of sampling it is therefore very difficult to determine treatment effects on ovarian function.

## 6.5 CONCLUSION

The results of this study indicate that during lactation in the sow dopamine agonist administration is able to increase LH secretion after chronic but not acute treatment. This effect may be mediated by a disinhibition by dopamine of the opioid-inhibitory effect on LH secretion. Finally, prolactin per se does not directly influence LH secretion during lactation in the sow.

**Fig. 6.1** Individual plasma LH profiles during sampling periods at day 12, 19 and 26 of lactation in two sows treated with Cabergoline (CAB) and two control sows (CONT).


CAB
LH(ng/ml)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
12
19
26
CAB
LH(ng/ml)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
12
19
26
DAY OF LACTATION

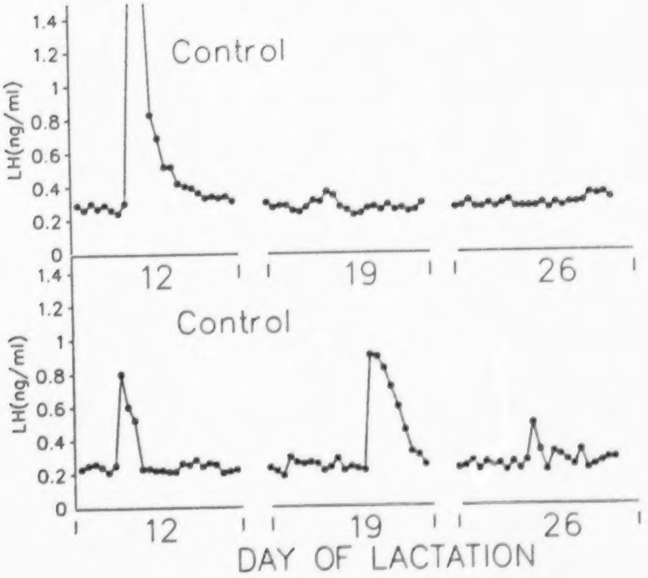
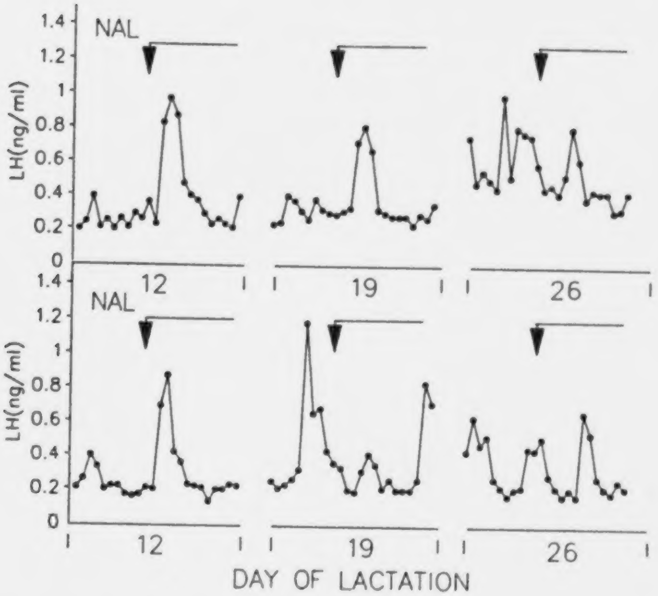

Control
LH(ng/ml)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
12
19
26
Control
LH(ng/ml)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
12
19
26
DAY OF LACTATION

**Fig. 6.2** Individual plasma LH profiles during sampling periods at day 12, 19 and 26 of lactation in two sows treated with Cabergoline and Naloxone (CAB+NAL) and two sows treated with Cabergoline and Morphine (CAB+MORPH).


NAL
NAL
LH(ng/ml)
LH(ng/ml)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
12
19
26
DAY OF LACTATION

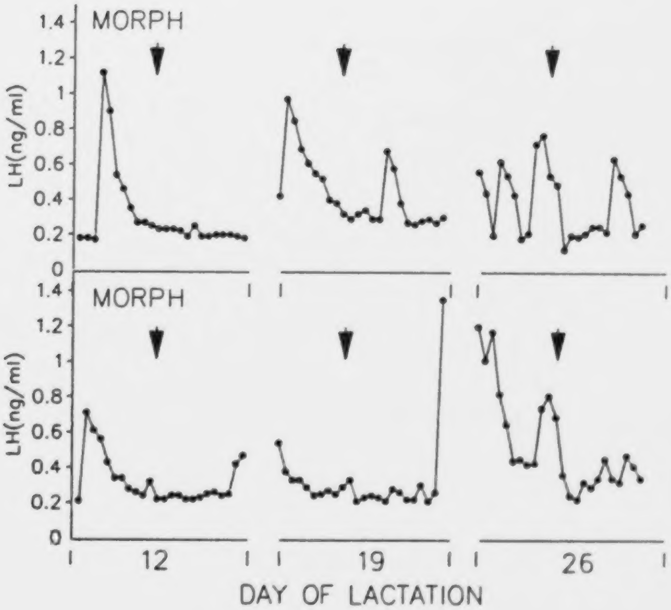

MORPH
MORPH
LH(ng/ml)
LH(ng/ml)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
12
19
26
DAY OF LACTATION

**Fig. 6.3a** Mean (+SEM) plasma LH in the sows treated with Cabergoline (CAB group) and in Control sows (CONT group). Each bar indicates the means for 3h-periods of sampling. Means with different superscripts differ ($p<0.05$).

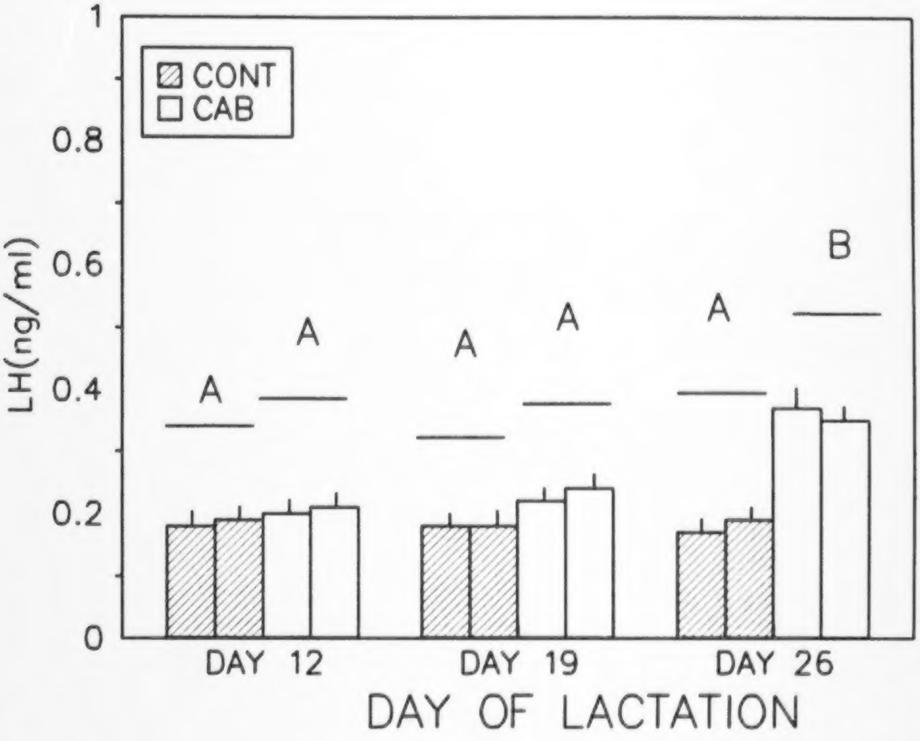
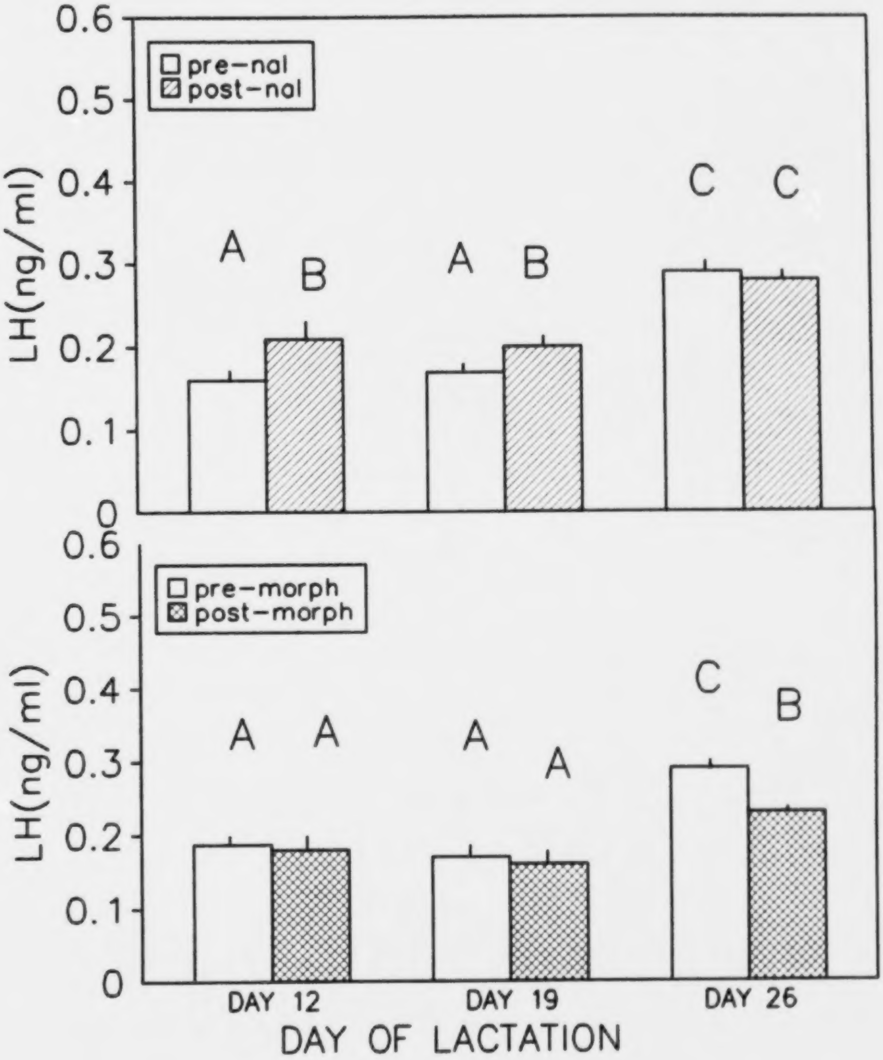

1
0.8
0.6
0.4
0.2
0
LH(ng/ml)
▨ CONT
☐ CAB
A
A
A
A
A
B
DAY 12
DAY 19
DAY 26
DAY OF LACTATION

**Fig. 6.3b** Mean (+SEM) plasma LH in the sows treated with Cabergoline and Naloxone (CAB+NAL group) and in the sows treated with Cabergoline and Morphine (CAB+MORPH group). Each bar indicates the means for 3h-periods of sampling. Means with different superscripts within treatments differ ($p<0.05$).



0.6
0.5
0.4
0.3
0.2
0.1
0
LH(ng/ml)
pre-nal
post-nal
A
B
A
B
C
C
0.6
0.5
0.4
0.3
0.2
0.1
0
LH(ng/ml)
pre-morph
post-morph
A
A
A
A
C
B
DAY 12
DAY 19
DAY 26
DAY OF LACTATION

**Fig 6.4** Individual plasma prolactin profiles of two control sows (CONT group) during the sampling period at day 12, 19 and 26 of lactation.

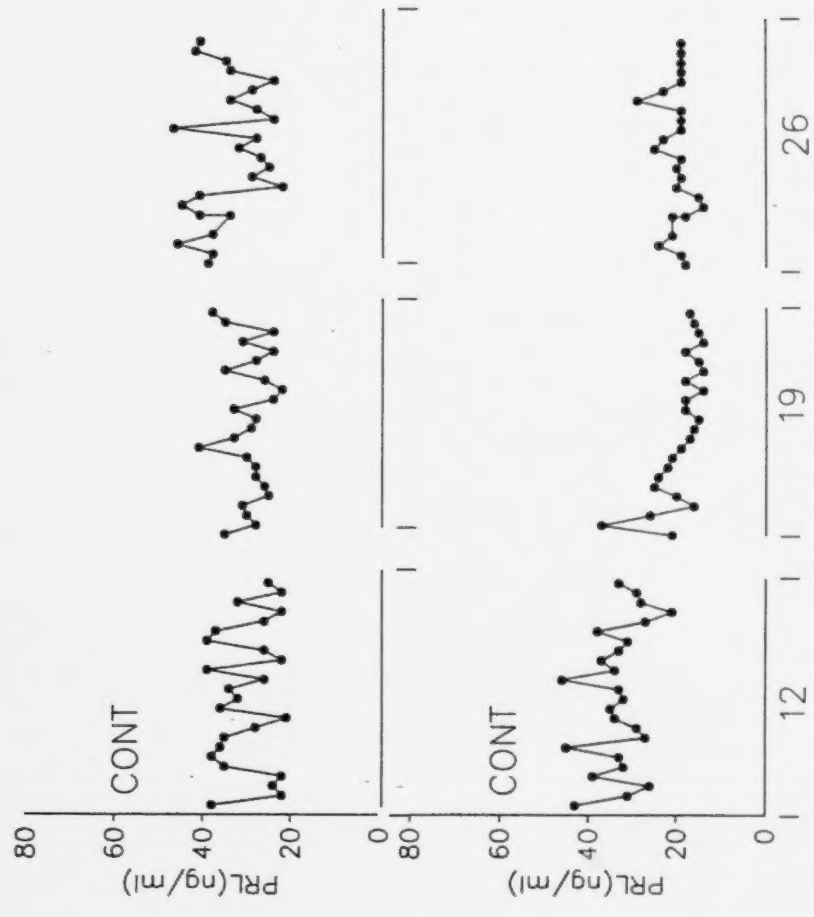

PRL(ng/ml)
80
60
40
20
0
CONT
PRL(ng/ml)
80
60
40
20
0
CONT
12
19
26
DAY OF LACTATION

**Fig 6.5** Individual plasma prolactin profiles of two sows treated with cabergoline (CAB group) during the sampling period at day 12, 19 and 26 of lactation.

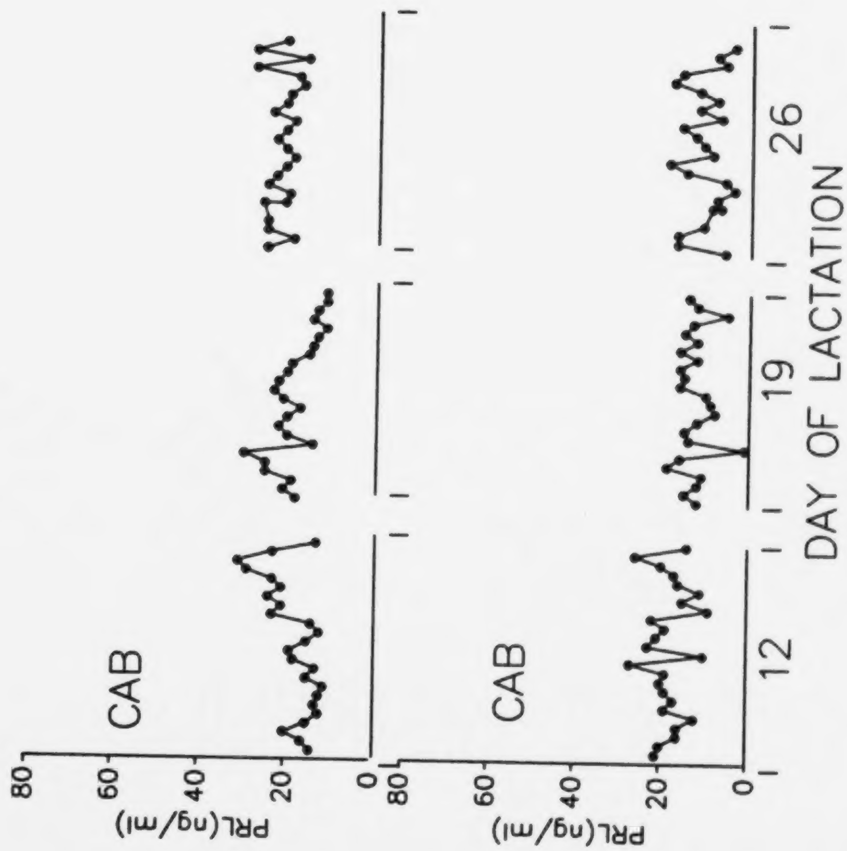

CAB
PRL (ng/ml)
80
60
40
20
0
CAB
PRL (ng/ml)
80
60
40
20
0
12
19
26
DAY OF LACTATION

**Fig 6.6** Individual plasma prolactin profiles of one sow treated with Cabergoline and Naloxone (CAB+NAL) and one sow treated with Cabergoline and Morphine (CAB+MORPH) during the sampling period at day 12, 19 and 26 of lactation. Arrows plus horizontal bars indicate naloxone treatment, single arrows indicate morphine treatment.

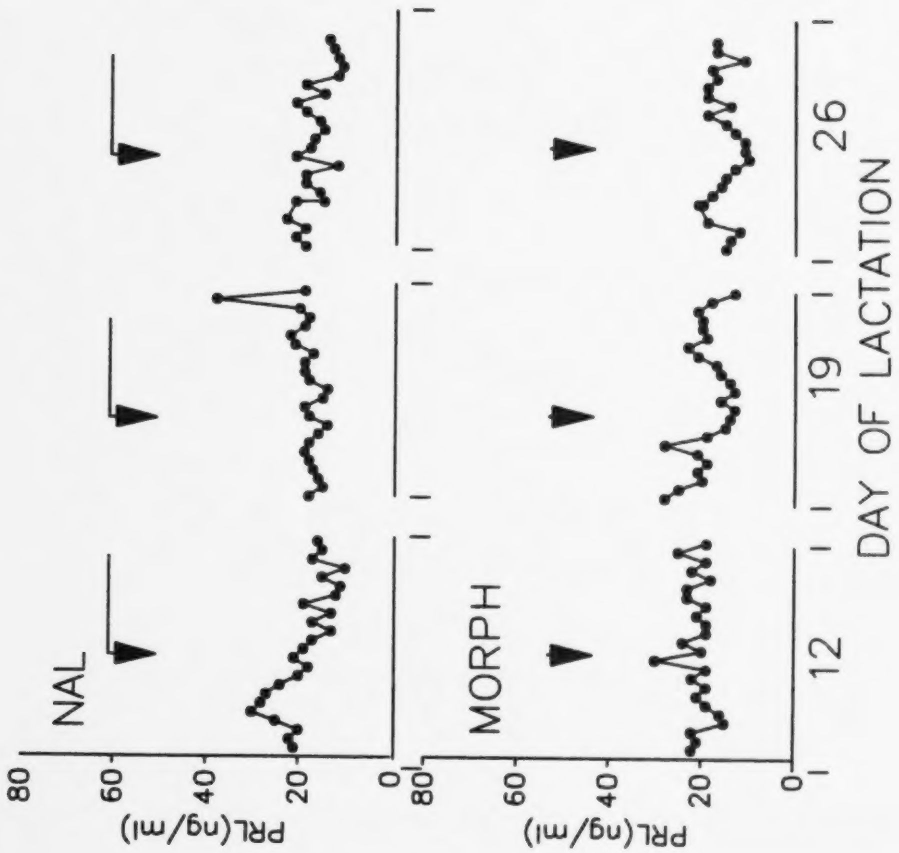
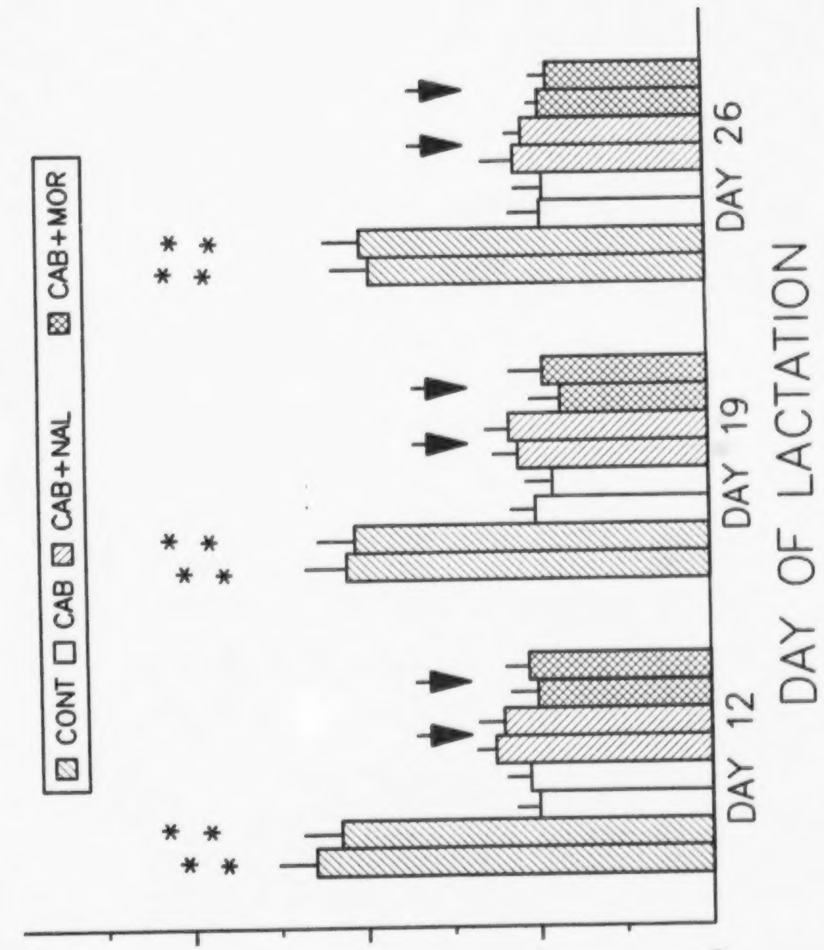

NAL
MORPH
PRL(ng/ml)
PRL(ng/ml)
80
60
40
20
0
80
60
40
20
0
12
19
26
DAY OF LACTATION

**Fig. 6.7** Mean (+SEM) plasma PRL in CONT, CAB, CAB+NAL and CAB+MORPH groups. Each bar indicates the means for 3h-periods of sampling. Arrows indicate treatment with naloxone or morphine. **=p<0.001.


PRL(ng/ml)
80
60
40
20
0
▨ CONT
☐ CAB
▨ CAB+NAL
▩ CAB+MOR
DAY 12
DAY 19
DAY 26
DAY OF LACTATION
**
**

## 6.6 BIBLIOGRAPHY

Andersen, A.N., Hagen, C., Lange, P., Boesgaard. S., Djursin, H., Eldrup, E., Micic, S. (1987). Dopaminergic regulation of gonadotrophin levels and pulsatility in normal women. Fert. Steril. 47:391-395.

Armstrong, J.D., Kraeling, R.R. and Britt, J.H. (1988a). Morphine suppresses luteinizing hormone concentrations in transiently weaned sows and delays onset of oestrus after weaning. J. Anim. Sci., 66:2216-2222.

Armstrong, J.D., Kraeling, R.R, Britt, J.H. (1988b). Effects of naloxone or transient weaning on secretion of LH and prolactin in lactating sows. J. Reprod. Fertil., 83:301-308.

Autelitano, D.J., Clements, J.A., Nikolaidis, I., Canny, B.J., Funder, J.W. (1987). Concomitant dopaminergic and glucocorticoid control of pituitary proopiomelancortin messenger ribonucleic acid and beta-endorphin levels. Endocrinology., 121:1689-1693.

Barb, C.R., Kraeling, R.R., Rampacek, G.B., Whisnant, C.S. (1986). Opioid inhibition of luteinizing hormone secretion in the post-partum lactating sow. Biol. Reprod., 35:368-371.

Beck, W., Engelbart, S., Gelato, M., Wuttke, W. (1977). Antigonadal effect of prolactin in adult castrated and in immature female rats. Acta Endocrinol., 84:62-71.

Ben-Jonathan, N., Hyde, J.F., and Murai, I., (1988). Suckling-induced rise in prolactin: Mediation by prolactin-releasing factor from posterior pituitary. Int. Union. Physiol. Sci./Am. Physiol. Soc. NIPS, 31:172-175.

Benedetti, M.S., Dostert, F., Barone, D., Efthymiopoulos, C., Peretti, G,. Roncucci, R. (1990). In vivo interaction of cabergoline with rat brain dopamine receptors labelled with [3H]N-n-propylnorapomorphine. European J. of Pharmacol. 187:399-408.

Besser, G.M., Parkes, L., Edwards, C.R.W., Forsyth, I.A., McNeilly, A.S. (1972). Galatorrhea: successful treatment with reduction of plasma prolactin levels by bromoergocryptine. Br. Med. J., 3:669-671.

Bevers, M.M., Willemse, M.H., Kruip, T.A.M. (1983). The effect bromocriptine on luteinizing hormone levels in the lactating sow: evidence for a suppressive action by prolactin and the suckling stimulus. Acta Endocrinol., 104:261-265.

Boesgaard, S., Hagen, C., Hangaard, J., Andersen, A.N., Eldrup, E. (1991). Pulsatile gonadotrophin secretion and basal PRL levels during dopamine D1 receptor stimulation in normal women. Fert. Steril., 55:281-286.

Casy, A.F., Parfitt, R.T. (1986). Opioid Analgesics, chemisry and receptors. In: Opioid Analgesics. Plenum Press, New York, pp. 333-337.

Dailey, R.A., Deave, D.R., Goodman, R.L. (1987). Neurotransmitter regulation of LH and PRL secretion. J. Reprod. Fertil., Suppl. 34:17-26.

Deaver, D.R., Dailey, R.A. (1982). Effects of dopamine, norepinephrine and serotonin on plasma concentrations of luteinizing hormone and prolactin in ovariectomized and anestrous ewe. Biol. Reprod. 27:624-632.

Deaver, D.R., Keisler, D.H., Dailey, R.A. (1987). Effects of domperidone and thyrotropin-releasing hormone on secretion of LH and PRL during the luteal phase and following induction of luteal regression in sheep. Dom. Anim. Endocrinol., 4:94-102

Del Pozo, E., Varga, L., Wyss, H., Tolis, G., Freisen, H., Wenner, R., Vetter, L., Uttewiler, A. (1974). Clinical and hormonal response to bromocriptine (CB 154) in the galactorhea syndromes. J. Clin. Endocrinol. Metab., 39:18-21.

Demarest, K.T., McKay, D.W., Riegle, G.D., Moore, K.E. (1983). Biochemical indices of tuberoinfundiular dopaminergic neuronal activity during lactation: a lack of response to prolactin. Neuroendocrinol., 32:24-27.

De Rensis, F., Foxcroft, G.R. (1993a). Endogenous opioid modulation of gonadotropin and prolactin secretion in the post-partum sow. In: Opioids in farm animals, Ed. N.Parvizi, Landwirtschaftsverlag GmbH, 4400 Münster-Hiltrup, Germany. pp. 15-33.

De Rensis F., Hunter, M., Foxcroft, G.R. (1993b). Suckling-induced inhibition of LH secretion and follicular development in the early post-partum sow. Biol. Reprod., 48:963-970

De Rensis, F., Cosgrove, J., Foxcroft, G.R. (1993c). Luteinizing hormone and prolactin responses to naloxone vary with stage of lactation in the sow. Biol. Reprod, 48:970-976.

Di Salle, E., Ornati, G., Giudici, D., Ragazzi P. (1983). J. Endocrinol. Invest. 6 (suppl.1).

Djursing, H., Hagen, C., Christensen, F., Nickelsen, C., (1981). Bromocriptine and estrogen modulation of gonadotropin release in normo- and hyperprolactinaemic patients with amenorrhea. Clin. Endocrinol., (Oxf.) 15:125-132.

Drouva, S.V. and Gallo, R.V. (1976). Catecholaminergic involvement in episodic luteinizing hormone release in ovariectomized rats. Endocrinology., 99:651-658.

Evans, W.S., Rogol, A.D., McLeod, R.M., Thorner, M.O. (1980). Dopaminergic mechanism and luteinizing hormone secretion. I. Acute administration of dopamine agonist bromocriptine does not inhibit luteinizing hormone release in hyperprolactinemic women. J. Clin. Endocrinol. Metab., 50:103-107.

Farah., J.M., Sapum-Malcom, D., Muller, G.P. (1983). Dopaminergic inhibition of pituitary ß-endorphin-like immunoreactivity secretion in the rat. Endocrinology., 110:657-659.

Findell, P.R., Wong, K.H., Jackman, J.K., Daniels, D.V. (1993). Beta1-adrenergic and dopamine (D1)-receptors coupled to adenylyl cyclase activation in GT1 gonadotropin-releasing-hormone neurosecretory cells. Endocrinology., 132:682-688.

Foxcroft, G.R., Haresign, N.B., Haynes, N.B., Lamming, G.E., Peters, A.R. (1988). Gonadotropins-Domestic Animals. Acta Endocrinol., 288:41-50.

Genazzani, A.R., Petraglia, F. Nappi, G., Martignoni, E., De Leo, M., Facchinetti, F. (1984). Endorphins in peripheral plasma:origin and influencing factors. In: Central and peripheral endorphins: Basic and clinical aspects. Ed. E.E. Muller, A.R. Genazzani, Raven press, New York. pp. 89-96.

Giraud, P., Lissitzky, C., Devolix, B.C., Gilloz, P., Oliver, C. (1980). Influence of haloperidol on ACTH and ß-endorphin secretion in the rat. Eur. J. Pharmacol., 62:215-217.

Hansen, S., Sodersten, P., Emeroth, P. (1983) Mechanisms regulating hormone release and the duration of dioestrus in lactating rat. J. Endocrinol., 99:173-180.

Jackson, G.L. (1977). Effect of adrenergic blocking drugs on secretion of LH in the ovariectomized ewe. Biol Reprod., 16:543-548.

Jori, M.C., Franceschi, M.C., Giust, N., Canal, R., Piolti, L., Frattola, S., Bassi, E., Galloni, E., Mamoli, A., Camerlingo, M. (1987). Cabergoline, a new ergoline derivate with long lasting dopaminergic properties. Tal. J. Neurol. Sci., 5, Suppl. 7:61

Kazmi, S.M.I., Mishra, R.K. (1987). Comparative pharmacological properties and functional coupling of μ and δ opioidreceptor sites in human blastoma SH-SY5Y cells. Mol. Pharmacol., 32:109-118.

Khatchaturian, H., Allesi, N.E., Lewis, M.E., Munfakh, N.,Fitzsimmons, M.D., Watson, S.J. (1985). Development of hypothalamic opioid neurons: a combined immunocytochemical and [3H]-thymidine autoradiographic study. Neuropeptides, 5:477-480.

Kraeling, R.R., Rampacek, G.B., Cox, N.M., Kiser, T.E. (1982). Prolactin and luteinizing hormone secretion after bromocriptine (CB 154) treatment in lactating sows and ovariectomized gilts. J. Anim. Sci., 54:1212-1220.

Kremer, J.A.M., Borm, G., Schellekens, L.A., Thomas, C.M.G., Rune, R. (1991). Pulsatile secretion of luteinizing hormone and prolactin in lactating and non lactating women and the response to naltrexone. J. Clin. Endocrinol. Metab., 72:294-300.

Kuljis, R.O., Advis, J.P. (1989). Immunocytochemical and physiological evisennce of a synapse between dopamine and luteinizing hormone releasing hormone-containing neurons in the ewe median eminence. Endocrinology., 124:1579-1581.

Laedem, C.A., Kalra, S.P. (1985). Effects of endogenous opioid peptides and opiates on LH and prolactin secretion. Neuroendocrinol., 41:342-352.

Leebaw, W.F., Lee, L.A., Woolf, P.D. (1978). Dopamine effects on basal and augmented pituitary hormone secretion. J. Clin. Endocrinol. Metab., 47:480-487.

Leibowitz, S.F., Jhanwar-Uniyal, M., Buorkin, B., Mackman,M.H. (1982). Distribution of alfa-adrenergic, beta-adrenergic and dopamine receptor in discrete nhypothalamic areas of the rat. Brain Res., 233(1):97-114.

Lehman, M.N., Karsh, F.J., Silverman, A.J. (1988). Potential site of interaction between catecholamine and LHRH in sheep brain. Brain. Res. Bull., 20:49-58.

Lord, J.A.H., Waterfeld, A.A., Hughes, J., Kosterlitz, H.W. (1977) Endogenous opioid peptide: multiple agonist and receptors. Nature, 267:495-499.

Maffeo, G., De Rensis, F., Vigo, D., Ballabio, R. (1989). Effetto della cabergolina sulla secrezione lattea e sulla ripresa dell'attivita' ciclica ovarica dopo lo svezzamento nella specie suina. Riv. Zoot. Vet., 17, n1, 3-11.

Marchetti, B., Labrie, F. (1982). Prolactin inhibits pituitary luteinizing hormone-releasing hormone receptors in the rat. Endocrinology, 111:1209-1216.

Martin, W.H., Rogol, A.D., Kaiser, D.L., Thorner, M.O. (1981). Dopaminergic mechanisms and luteinizing hormone (LH) secretion. II Differential effects of dopamine and bromocriptine on LH release in normal women. J. Clin. Endocrinol. Metab., 52:650-656.

Martinez-de-la-Escalera, G., Gallo, F., Choi, A.L., Weiner, R.I. (1992) Dopaminergic regulation of the GT1 gonadotropin-releasing hormone (GnRH) neuronal cell lines;

stimulation of GnRH release via D1-receptors positively coupled to adenylate cyclase. Endocrinology., 131:2965-71.

Mattioli, M., Seren, E. (1985). Effect of bromocriptine treatment during lactational anestrous in the pig. In: Endocrine causes of seasonal and lactational anestrous in farm animals. Ed. Ellendorff, F., Elasaesser, F., Martinus-Nijhoff Pub., pp. 165-178.

Mattioli, M., Conte, F., Seren, E., Galeati, G. (1986). Effect of naloxone on plasma concentrations of prolactin and LH in lactating sows. J. Reprod. Fertil., 76:167-173.

McNeilly, A.S. (1987). Prolactin and the control of gonadotrophin secretion. J. Endocrinol., 115:1-5.

McNeilly, A.S. (1988). Suckling and the control of gonadotrophin secretin. In:The physiology of reproduction. Eds. E. Knobil, J.D. Neil. New York, Raven Press. pp 2323-2349.

Melis, G.B., Cagnacci, A., Paoletti, A.M., Caffi, T. (1988). Chronic bromocriptine administration restores luteinizing hormone response to naloxone in post-menopausal women. Neuroendocrinol., 47:159-163.

Meyer, S.l., Goodman, R.L. (1985). Neurotransmitters involved in mediating the steroid dependent suppression of pulsatile LH secretion in anestrous ewes; effects of receptor antagonists. Endocrinology., 116:2054-2161.

Miyachi, V., Meklenburg, R.S., Lipsett, M.B. (1973). In vitro studies of pituitary median eminence unit. Endocrinology., 93:492-496.

Moult, P.J.A., Rees, L.H., Besser, G.M. (1982). Pulsatile gonadotropin secretion in hyperprolactinaemic amenorrhea and the response to bromocriptine therapy. Clin Endocrinol., (Oxf.), 16:153-162.

Muralidhar, K., Maneckjee, R., Moudgal, N.R. (1977). Inhibition of in vivo pituitary release of luteinizing hormone in lactating rats by endogenous prolactin. Endocrinology, 100:1137-1142.

Panerai, A.E., Sawynok, J., La Bella, F.S., Friesen, H.G. (1980). Prolonged hyperprolactinemia influences beta-endorphin and metenkephaline in the brain. Endocrinology, 106:1804-1808.

Pasternak, G.W. (1986a). Multiple mu opiate receptors: biochemical and pharmacological evidence for multiplicity. Bioch. Pharmacol., 35:361.

Peters, A.R., Lamming, G.E., Fisher, M.W. (1981). A comparison of plasma LH concentrations in milked and suckling post-partum cows. J. Reprod. Fertil., 62:567-573.

Pontiroli, A.E., Viberti G.C., Mangili, R., Cammelli, L., Orlandini, G., Pozza, G., Dubuni, A. (1985). Long lasting prolacting-lowering effect of FCE 21336 in man. J. Endocrinol. Invest., Suppl.3 8:103.

Ramirez, V.D., Feder, H.H., Sawyer, C.H. (1984). The role of brain catecholamines in the regulation of LH secretion: a critical inquiry. Front. Neuroendocrinol., 8:27-83.

Rasmussen, D.D., Liu, J.H., Wolf, P.L., Yen, S.S.C. (1986). GnRH neurosecretion in the human hypothalamus. In vitro regulation by dopamine. J. Clin. Endocrinol. Metab., 62:479-483.

Rotstzejin, W.H, Charli, J.L., Pattou, E., Kordon, C. (1977) Stimulation by dopamine of LHRH release from mediobasal hypothalamus in male rat. Endocrinology, 101:1475-1481.

Sarkar, D.K., Yen, S.S.C. (1985). Hyperprolactinemia decreases LH RH concentration in pituitary portal plasma: a possible role for beta-endorphin as mediator. Endocrinology., 116:2080-2084.

SAS. (1985) Sas User's Guide: Statistics Cary, NC: Statistical Analysis System Institute, Inc., Cary..

Schoors, D.F., Vauquelin, G.P., De Vos, H., Smet, G., Velkeiner, B., Vanhaaelst, L., Dupont, A.G. (1991). Identification of a D1 receptor, not linked to adenyl;ate cyclase, on lactotroph cells. Br. J. Pharmacol. 103:1928-1934.

Sharp, B., Karson, B., Marrshak, D., Ross, R., George, R., Sowers, J., Yamada, T. (1982). Life Sci. 31:981-985.

Shaw, H.J, Foxcroft, G.R., (1985). Relationship between LH, FSH, and prolactin secretion and reproductive activity in the weaned sow. J. Reprod. Fertil., 75:17-28.

Smith, S.M. (1978). The relative contribution of suckling and prolactin to the inhibition of gonadotrophin secretion during lactation in the rat. Biol. Reprod., 19:77-83.

Sneider, H.P.G. and McCann, S.M. (1970). Mono- and indolamines and control of LH secretion. Endocrinology., 86:1127-1133.

Ssewannyyana, E., Lincoln, G.A. (1990). Regulation of the photopheriod-induced cycle in the peripheral blood concentrations of beta-endorphin and prolactin in the ram: role of dopamine and endogenous opioids. J. Endocrinol., 127:461-469.

Thokac, M.D., Pettigrew, J.E., Dial, G.D., Wheaton, J.E., Crooker, B.A., Johnston, L.J. (1992). Characterization of LH secretion in the promiparous lactating sow: relationship to blood metabolites and return to estrus interval. J. Anim. Sci., 70:2195-2201.

Thorner, M.O., Besser, G.M., Jones, A., Dacie, J., Jones, A.E. (1975). Bromocriptine therapy of female infertility-a report of 13 pregnancies. Br. Med. J., 4:649-651.

Tolis, G., Pinter, E.J., Friesen, H.G. (1975). The acute effect of 2-bromocriptine (CB-154) on anterior pituitary hormones and free fatty acid in man. Int. J. Clin. Pharmacol., 12:281-283.

Uemura, H., Kobayashi, H. (1971). Effects of dopamine implanted in the median eminence on the oestrus cycle in the rat. Endocrinol. Jap., 18:91-94.

Van Vugt, D.A., Meites, J. (1980) Influence of endogenous opiates on anterior pituitary function. Fed. Proc., 39:2533-2538.

Vermes, I., Tilders, F.J.H., Maysinger, (1984). Release of opioid peptides from rat hypothalamus in vitro: dopamine-receptor mediated inhibition of beta-endorphine release. In: Central and peripheral endorphins: Basic and clinical aspects. Ed. by E.E. Muller and A.R. Genazzani, Raven Press, New York. pp. 109-113.

Weiner, R.I., Findell, P.R., Kordon, (1988). Role of classic and peptide neuromediators in the neuroendocrine regulation of LH and prolactin secretion. In: The Physiology of reproduction. Ed. by E. Knobil, J.D. Neill, pp. 1349-1378.

# CHAPTER 7

## 7.1 DISCUSSION

As reviewed in the introduction of the present thesis the endocrinology of the sow during lactation is characterized by:

1) Low FSH concentrations in early lactation which tend to increase with time from farrowing (Stevenson et al., 1981; Edwards et al.,1983; De Rensis et al., 1991). However, these concentrations of FSH are able to maintain a follicular cohort for subsequent development (De Rensis et al., 1991). The suppression of FSH secretion is known to involve a non-steroidal ovarian regulator (Stevenson et al., 1981; Cox and Britt, 1982b) and FSH secretion in lactation requires minimal GnRH stimulation (Chappel et al., 1983).

2) Low LH concentrations during established lactation in the sow (Parvizi et al., 1976; Stevenson et al., 1981; Shaw et al., 1985; Foxcroft et al., 1987; De Rensis et al., 1991). An inhibitory effect of suckling on LH secretion appears some hours after farrowing (De Rensis et al., 1993b) and seems proportional to the numbers of mammary quarters suckled by piglets and to the neural intensity of the suckling stimulus (Grant, 1989). This inhibitory effect decreases during later lactation as suckling frequency and milk production declines and this results in a gradual increase of circulating LH with time after farrowing (see Britt et al., 1985). The changes in LH secretion in established lactation are evident as modulations of a very characteristic low frequency, high amplitude pattern of episodic release. Episodic frequency increases as lactation progresses and is higher in sows returning to estrus soon after weaning (Shaw and Foxcroft, 1985: Foxcroft et al., 1987).

3) An absence of GnRH secretion. Evidence that the suckling stimulus inhibits GnRH secretion is provided by recent studies examining the effect of N-methyl-D,L aspartic acid (NMA) to trigger release of LH during lactation in the sow, thereby demonstrating that suckling-induced suppression of LHRH turnover was limiting LH secretion during lactation (Sesti et al.,1993). Single (Bevers et al., 1981; Mattioli et al., 1985; Rojanasthien et al.,1987) or pulsatile (Guthrie et al.,1978; Cox et al., 1987; Rojanasthien et al., 1987; De Rensis et al.,1991) GnRH administration results in a substantial release of plasma LH at all stages of lactation and pulsatile GnRH injection can induce estrus and ovulation during lactation.

4) High plasma prolactin after farrowing which gradually declines with time during lactation (Stevenson et al., 1981; De Rensis et al., 1991,1993b). Several lines of evidence suggest that suckling increases PRL secretion by a brief removal of dopaminergic inhibition, stimulation of PRF release and/or increased lactotrope responsiveness to the action of PRF (Plotsky et al., 1992).

5) Suppression of ovarian follicular development, characterized by a large population of small-sized follicles, a small population of medium-sized follicle and a few large sized

follicles (Palmer et al., 1965; Kunavongkrit et al 1982; Dyck, 1983; Grant et al., 1989).

6) Low (Smith et al., 1992) or variable (Varley et al., 1990) plasma estradiol-17beta. However, estradiol concentrations are related to the reproductive performance of each sow. Suckling changes the sensitivity of the hypothalamic-pituitary axis to estradiol feedback and both inhibitory and stimulatory effects on LH have been described, depending on the stage of lactation considered and the treatment used (Elsaesser et al.,1980; Ramirez et al.,1985; De Rensis et al., 1991: Sesti et al., 1989).

In conclusion, since there is clear evidence that pulsatile LH release occurs in response to the pulsatile secretion of GnRH from the hypothalamus (Clarke et al., 1982) and GnRH treatment is able to induce follicular development (Guthrie et al., 1987; Cox and al., 1982a; Rojanasthien et al., 1987; De Rensis et al.,1991) during lactation in the sow, the main factor that inhibits follicular development and thus reproductive activity during lactational anestrus is the suppressive effect of the suckling stimulus on the hypothalamic release of GnRH.

It is known that afferent fibres from the nipple and mammary gland travel via the mammary nerve to the spinal cord and that these axons ascend largely uncrossed in the dorsal and lateral columns into the brain stem. Then it appears that the "suckling" axons follow a diffuse and perhaps, multisynaptic path throughout the reticular formation, enter the hypothalamus via diverse routes and terminate in several different hypothalamic nuclei involved in the regulation of GnRH, PRL and oxytocin secretion.

The identification of the substances which transmit signals from the midbrain to the hypothalamus and coordinate events within the hypothalamus has become a central issue of GnRH\LH research. In the present thesis the hormonal mechanisms that mediate the effects of the suckling stimulus on GnRH and thus LH secretion during lactation in the pig were investigated. Since a close correlation exists between suckling and prolactin, this hormone was also investigated for a potential role in the suppression of GnRH/LH release.

The studies of this thesis developed the results of a previous experiment in which the relationships among LH, FSH and PRL secretion and follicular development in lactating and zero-weaned sows were investigated (De Rensis et al., 1993b). The results showed that the suckling stimulus was not inhibiting LH secretion during the first 66-78h after farrowing. In contrast, in sows weaned at birth, active LH secretion was maintained beyond this time and was associated with immediate follicular development. However, from the results of this study it was not possible to identify which factors were inhibiting LH secretion 66-78h post-partum in suckled sows. The literature indicated that exogenous GnRH treatment could override suckling-induced inhibition of LH secretion and that the opioidergic system was one of the major mechanisms mediating the effects of suckling (Barb et al.,1986; Mattioli et al.,1986). Therefore the first three experiments of this thesis were developed to describe the involvement of the opioidergic system in the control of GnRH\LH and PRL secretion during different periods of lactation in the sow. In experiment four, these results were extended to demonstrate that other factors together with the opioids, are involved in the inhibition of LH secretion during lactation. In the fifth and final experiment the possibility that one of these "other factors" is the dopaminergic system was studied. As an integral part of these experiments the opioidergic control of PRL secretion and the involvement of PRL in the control of reproduction during lactation was studied.

The presence of active LH secretion immediately after farrowing as observed in previous studies (Tokach et al., 1992; Sesti et al., 1991; De Rensis et al.,1993b) and in experiment 1 in this thesis was not confirmed in later studies of this thesis (experiments 2; 3 and 4). This could be due to the fact that in the majority of the animals in the later experiments LH secretion was inhibited very early post-partum, as was observed in some animals in the previous studies (De Rensis et al., 1993b) and in experiment 1. In this context it is relevant to emphasize that the genetic origin of the sows used changed as the research program developed. Originally terminal line gilts (Camborough x Canabrid) from the University herd were used. More recently, dam-line replacement gilts from a multiplier unit were used. Also it should be emphasized that the bleeding schedule adopted in experiments 2, 3 and 4 did not require blood sampling until 24-36h after parturition. Generally however, considering all the experiments together it seems that the length of the period of active LH secretion immediately after farrowing is subject to great variability between animals and can be very short in some animals. However, in relation to the hypotheses under consideration, this earlier suckling-induced inhibition of LH secretion was still associated with an absence of any clear effect of opioidergic regulation until about 72h after farrowing.

The presence of individual sows that did not respond to naloxone injection during established lactation merits particular discussion. Different hypotheses can be advanced to explain this observation and indeed, an understanding of the causes of a lack of an effect of naloxone on LH secretion during lactation in some animals could be a key for a better understanding the role of the opioids in inhibiting LH secretion. One possibility is that the dose of naloxone used was insufficient to antagonize the opioidergic tone in some sows. The results of part one of the first experiment (De Rensis et al., 1993b) in part support this possibility. We observed that 72h post-partum, there is a dose-dependent elevation in LH secretion. However, the studies of Barb et al. (1986) suggest a threshold for an LH response at day 22 of lactation of 1mg/kg of naloxone. Therefore the 2mg/kg dose utilized in our experiments should be effective in overcoming an existing opioidergic inhibitory tone. Thus we consider that this hypothesis can be rejected.

Another possibility is the absence of a releasable GnRH pool at the moment of the treatment. However, in the sows with a lack of an immediate response to opioid antagonist, more active secretion of LH during the later period of sampling would be expected because of the absence of inhibitory opioidergic tone. Therefore, the possibility of an absence of a releasable LH pituitary pool can be excluded. Also, as in the GnRH experiment and other previous studies (Cox and Britt, 1982a; Rojanasthien et al., 1987; De Rensis et al., 1991) all the sows responded to exogenous GnRH treatment in early as well as in mid and late lactation.

The possibility that the absence of a LH response to naloxone could be related to high cortisol levels due to the experimental conditions, as suggested for the lactating cow (Whisnant et al., 1986) cannot be totally excluded. However, we believe that such stressful conditions did not exist for the animals during these experiments. The jugular or cephalic cannulation utilized in these studies is a reliable method for stress-free sampling and modifications in the suckling behaviour of the sows studied was not observed.

Therefore a hypothesis involving effects of estradiol-17beta may be proposed. It has been well demonstrated that naloxone-induced increases in serum LH are related to the steroid milieu present (Bhanot et al., 1984; Gregg et al., 1986; Brooks et al., 1986; Currie et al., 1987; Whisnant et al., 1988; Currie et al., 1989). The naloxone-induced elevation of LH is greatly attenuated, or totally absent, in gonadectomized rats but can be restored by pretreatment with estrogens (Bhanot et al., 1983). Moreover, estrogen decreases the hypothalamic concentration of beta-endorphin (Wardlaw et al.,1982) and increases beta-endorphin release into the hypophyseal portal blood (Wardlaw at al., 1982; Sarkar and Yen, 1985). There is, however, the contradiction that steroid hormones are low and are generally assumed to play a minor role in the inhibition of reproductive activity throughout lactation; indeed ovariectomy in lactation had no effect on LH secretion (Stevenson et al., 1981). However, it has to be considered that the latter authors were analyzing the mean estradiol-17beta concentrations for all the animals studied, while, to explain an absence of a naloxone effect it is important to consider the profile of this steroid in the single animal. Varley and Atkinson (unpublished data, see Varley and Foxcroft, 1990) report that estradiol concentrations during lactation in the sow may not be low throughout lactation but can be extremely variable, with a tendency for an increase around three weeks post-partum. Also during lactation, there are pools of follicles that develop but which cannot reach maturity, due to the absence of sufficient LH activity. Therefore, it is possible that when a pool of follicles develops there is an increase in estradiol levels and thus an increase in the opioid inhibition of LH secretion and this situation can be reversed by naloxone treatment. Alternatively, when there are low amounts of circulating estradiol because of absence of any follicular development, no clear effect of naloxone can be detected. However, there are studies that indicate that a limited amount of estradiol can be secreted from the relatively small follicular structures seen in mid lactation (Edquist et al., 1974; Palmer et al., 1965). Another possibility is that accumulated fat stores retain significant quantities of lipophilic steroids and that, as a consequence of a body weight loss in lactation, some of these steroids could be released into the general circulation (Hillbrand and Elsaesser, 1983; Prime et al.,1988). Thus these steroid hormones would be released in different amounts depending on the catabolism of the animals and thereby influence the opioidergic effect on LH secretion and thus the naloxone disinhibition of LH secretion. In conclusion, all these mechanisms are strategies that can block follicular development during lactation. In the presence of initial follicular development and therefore steroid secretion, a steroid-dependent increase in opioidergic inhibition of the GnRH\LH axis would provide the block to further follicular development. Similarly, mobilisation of fat deposits during lactation in the sow might release steroid hormones and thereby increase the opioidergic inhibition of LH secretion, providing one mechanism by which tissue catabolism could be associated with an inhibition of reproductive activity.

A general scheme summarizing some of the factors that are involved in the control of LH secretion in the sow during lactation is shown in Fig. 7.1 and this also summarizes some of the results of this thesis. The post-partum period in the sow can be divided into three stages, one immediately after farrowing, the second during mid lactation (2-3 weeks) and the third in late lactation (3 weeks onwards). The first period immediately after farrowing, is characterized by active LH secretion. This situation is subject to high variability between animals and can be as short as 24h post-partum. This LH secretion could be the result of a balance between two effects, a stimulatory effect due to decreased levels of steroid hormones following farrowing and a residual inhibitory effect of the high steroid levels during pregnancy. Furthermore, the data from experiments 1, 2, and 3 indicate that the opioidergic system is not involved in the control of LH secretion during this stage of lactation. With regard to mid lactation, the results of this thesis indicate that there is a clear inhibitory effect of the opioidergic system on LH secretion. However, there are data suggesting that other systems could also be involved.

Finally, in late lactation in sows there is an "escape" of the hypothalamo-pituitary axis from the inhibitory effect of suckling. At this time the involvement of a dopaminergic system in the control of LH secretion could be observed by the chronic treatment with dopamine agonists.

In Fig. 7.2 potential exogenous treatments to overcome lactational anestrus are shown, as well as the period of lactation during which they could be effective in overriding the inhibitory effect of suckling. This figure therefore summarizes the main factors that determine the lack of follicular development during lactation.

## 7.2 CONCLUSION

In conclusion the results of these studies can be briefly summarized as following. 1) The opioidergic and other systems are involved in the control of LH and PRL secretion during established lactation but not during early post-partum period. 2) Factors apart from the opioidergic system are also involved in mediating the initial inhibitory effect of the suckling stimulus on gonadotropin secretion. 3) The opioids are not involved in the control of FSH secretion, therefore in part contributing to differential regulation of gonadotropin secretion during lactation. 4) The short-term administration of opioid antagonists does not modify follicular development during mid lactation. 5) PRL secretion during lactation in the sow is inhibited by the administration of both an opioid agonist (morphine) and a dopamine agonist (Cabergoline). 6) It is unlikely that PRL per se directly influences LH secretion during lactation in the sow.

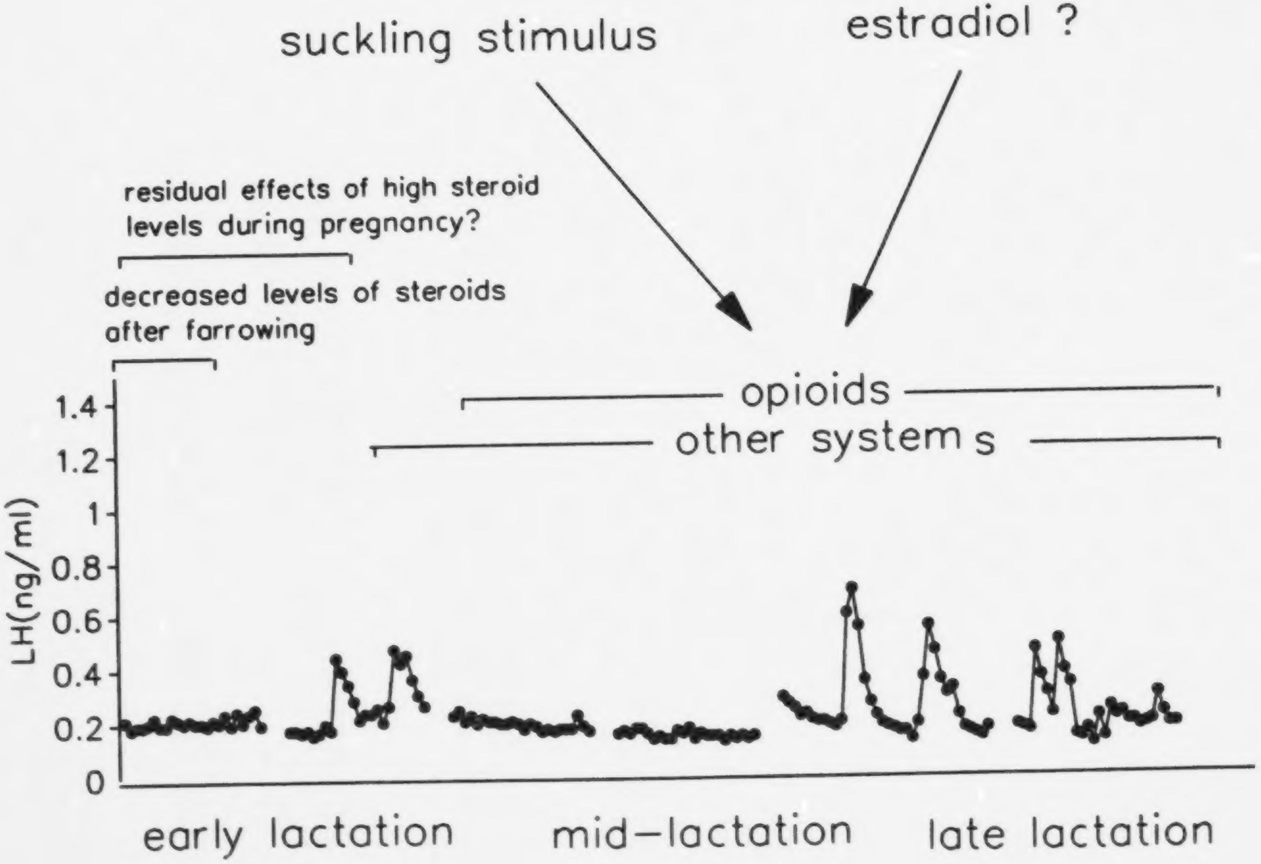
**Fig. 7.1** A general scheme summarizing some of the factors that are involved in the control of LH secretion in the sow during lactation.

## Factors that can induce follicular development during lactation in the sow

| EARLY LACTATION | MID LACTATION | LATE LACTATION |
|---|---|---|
| GnRH | GnRH | GnRH |
| xxxx | Opioid antagonists | Opioid antagonists |
| DA D1 receptor agonists? | DA D1 receptor agonists? | DA D1 receptor agonists? |
| xxxx | xxxx | DA D2 receptor agonists |
| E2 immunization | E2 immunization | E2 immunization? |
| noradrenergic agonists? | noradrenergic agonists? | noradrenergic agonists? |

DA = Dopamine
E2 = Estradiol-17beta
GnRH = Gonadotropin releasing hormone

**Fig. 7.2** Potential exogenous treatments to overcome lactational anestrus and the period of lactation during which they could be effective in overriding the inhibitory effect of suckling.


suckling stimulus
estradiol ?
residual effects of high steroid levels during pregnancy?
decreased levels of steroids after farrowing
opioids
other systems
LH(ng/ml)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
early lactation
mid-lactation
late lactation

# BIBLIOGRAPHY

Bevers, M.M., Willemse, M.H., Kruip, T.A.M., Van de Wiel D.F.M. (1981). Prolactin levels and the LH response to synthetic LHRH in lactating sow. Anim. Reprod. Sci., 4:155-163.

Bhanot, R., Wilkinson, M. (1984). The inhibitory effects of opiates on gonadotropin secretion is dependent upon gonadal steroids. J. Endocrinol., 102:133-141.

Britt, J.H., Armstrong, J.D., Cox, N.M., Esbenshade, K. (1985). Control of follicular development during and after lactation in the sow. J. Reprod. Fertil., 33:37-54

Brooks, A.N., Lamming, G.E., Lees, P.D., Haynes, N.B. (1986). Opioid modulation of LH secretion in the ewe. J. Reprod. Fertil. 76:689-708.

Chappel, S.C., Ulloa-Aguirre, A., Coutifaris, C. (1983). Biosynthesis and secretion of follicle-stimulating hormone. Endocr. Rev., 4:179-211.

Clarke, I.J., Cummins, J.T. (1982). The temporal relationships between gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) secretion in ovariectomized ewes. Endocrinology, 111:1737-1739.

Cox, N.M., Britt, J.H. (1982a). Pulsatile administration of gonadotropin releasing hormone to lactating sows: endocrine changes associated with induction of fertile estrus. Biol. Reprod., 27:1126-1137.

Cox, N.M., Britt, J.H. (1982b). Relationship between endogenous gonadotropin-releasing-hormone, gonadotropins, and follicular development after weaning in sows. Biol. Reprod., 27:70-78.

Cox, N.M, Armstrong, J.D., Britt, J.D. (1987). Influence of follicular ablation during lactation on postweaning interval to estrus, endocrine functions in sow. Dom. Anim. Endocrinol., 4:87-93.

Currie, W.D., Rawlings, N.C. (1989). Fluctuation in responsiveness of LH and lack of responsiveness of FSH to prolonged infusion of morphine and naloxone in the ewe. J. Reprod. Fertil., 86:359-366.

Currie, W.D., Rawlings, N.C. (1987). Naloxone enhances LH but not FSH release during various phases of the estrous cycle in the ewe. Life Sci., 41:1207-1214.

De Rensis, F., Hunter, M.G., Grant, S.A., Lancaster, R.T., Foxcroft, G.R. (1991). Effect of estrogen administration on endogenous and luteinizing-hormone-releasing-hormone-induced luteinizing hormone secretion and follicular development in the lactating sow.

Biol. Reprod., 44:975-982.

De Rensis, F., Foxcroft, G.R. (1993a). Endogenous opioid modulation of gonadotropin and prolactin secretion in the post-partum ewe. In: Opioids in farm animals, Ed. N.Parvizi, Landwirtschaftsverlag GmbH, 4400 Münster-Hiltrup, Germany. pp. 15-33.

De Rensis, F., Hunter, M.G., Foxcroft, G.R. (1993b). Suckling-induced inhibition of LH secretion and follicular development in the early post-partum sow. Biol. Reprod., 48:964-969.

De Rensis, F., Cosgrove, J., Foxcroft, G.R. (1993c). Luteinizing hormone and prolactin responses to naloxone vary with stage of lactation in the sow. Biol. Reprod., 48:970-976.

Dyck, G.W. (1983). Postweaning changes in the reproductive tract of the sow. Can. J. Anim. Sci., 63:571-577.

Edquist, L.E., Einarsson S., Settergren, I. (1974). Ovarian activity and peripheral plasma levels of oestrogens and progesterone in the lactating sow. Theriogenology, 1:43-49.

Edwards, S., Foxcroft, G.R. (1983). Endocrine changes in sows weaned at two stages of lactation. J. Reprod. Fertil., 67:161-172.

Elsaesser, F., Parvizi, N. (1980). Partial recovery of the stimulatory oestrogen feed back action on LH release during late lactation in the pig. J. Reprod. Fertil., 59:63-67.

Foxcroft, G.R. (1985). Follicular dynamics and its endocrine control in the sow. In: Follicular growth and ovulation rate in farm animals. Eds. J. Roche, D. O'Callaghan. Martinus Nijhoff Publishers, pp.191-205.

Foxcroft, G.R., Shaw, H.J., Hunter, M.G., Booth, P.J. and Lancaster, R.T. (1987). Relationship between luteinizing hormone, follicle-stimulating hormone and prolactin secretion and ovarian follicular development in weaned sows. Biol. Reprod., 36:175-191.

Grant, S.A., Hunter M.G., Foxcroft, G.R. (1989). Morphological and biochemical characteristics during ovarian follicular development in the pig. J. Reprod. Fertil. 86, 171-183.

Gregg, D.W., Moss, G.E., Hudgens, R.E., Malven, P.V. (1986). Endogenous opioid modulation of luteinizing hormone and prolactin in post-partum ewes and cows. J. Anim. Sci., 63:838-847.

Guthrie, H.D., Bolt, D.J., Kiracofe, G.M., Miller, K.F. (1987). Effect of charcoal-extracted porcine follicular fluid and 17b-estradiol on follicular growth and plasma gonadotropins in gilts fed a progesterone agonist altrenogest. J. Anim. Sci. 64, 816-826.

Guthrie, H.D., Pursel, V.G., Frobish, L.T. (1978). Attempts to induce conception in lactating sows. J. Anim. Sci., 47:1145-1151.

Hillbrand, F.W., Elsaesser, F. (1983). Concentration of progesterone in the backfat of pigs during the estrous cycle and after ovariectomy. J. Reprod. Fertil., 69:73-80.

Kunavongkrit, A., Einarsson, S., Settergren, I. (1982). Follicular development in primiparous lactating sows. Anim. Reprod. Sci., 5:47-56.

Mattioli, M., Seren, E. (1985). Effect of bromocriptine treatment during lactational anestrus in the pig. In: Endocrine causes of seasonal and lactational anestrus in farm animals. Eds. Ellendorff, F., Elsaesser, F., Martinus-Nijhoff Pub., pp. 165-178.

Palmer, V.M., Teague, H.S., Venzke, W.G. (1965). Macroscopic observations on the reproductive tract of the sow during lactation and early post weaning. J. Anim. Sci., 24:541-545.

Parvizi, N., Elsaesser, F., Smidt, D., Ellendorff, F. (1976). Plasma luteinizing hormone and progesterone in the adult female pig during the oestrous cycle, late pregnancy and lactation, and after ovariectomy and pentobarbitone treatment. J. Endocrinol., 69:193-203.

Plotsky, P.M., Neill, J.D. (1982). The decrease in hypothalamic dopamine secretion induced by suckling: comparison of voltametric and radioisotopic methods of measurement. Endocrinology, 110:691-696.

Prime, G.R., Varley, M.A., Symonds, H.W. (1988). The effect of food intake in lactation and early pregnancy on plasma progesterone concentration and prolificacy in multiparous sows. Anim. Prod. 44:499, Abstract.

Ramirez, J.L., Bennet, W.A., Cox, N.M. (1985). Effect of stage of lactation on estrous behaviour, LH and ovulation after administration of estradiol benzoate to sows. J. Anim. Sci., 61:406 Abstr..

Rojanasthien, S.A.M., Lundeheim, N., Einarsson, S. (1987). Luteinizing hormone response to different doses of synthetic gonadotrophin releasing hormone during early and late lactation in primiparous sows. Anim. Reprod. Sci., 13:299-307.

Sarkar, D., Yen, S.S.C. (1985) Hyperprolactinaemia decreases the Luteinizing-hormone-releasing-hormone concentration in pituitary portal plasma. A possible role of beta-endorphine as a mediator. Endocrinology, 116:2080-2084.

Sesti L.A.C., Britt, J.K. (1989). Estrus and LH feedback in response to estradiol and LHRH during mid or late lactation. In: Proceedings of the Third International Conference on Pig Reproduction. Nottingham, U.K. Abstract n.6.

Sesti, L.A.C., Britt, J.H. (1991). Agonist-induced release of GnRH, LH and FSH and their associations with basal secretion of LH and FSH in lactating sows. Biol. of Reprod., 44:105 (Abstr.).

Sesti, L.A., Britt, J.H. (1993). Influence of stage of lactation, exogenous LHRH, and suckling on estrus, positive feed-back of LH, and ovulation in sow treated with estrogen. J. Anim. Sci., 71:989-998.

Shaw, H.J., Foxcroft, G.R. (1985). Relationship between LH, FSH and prolactin secretion and reproductive activity in the weaned sow. J. Reprod. Fertil., 75:17-28.

Smith, C.A., Almond, G.W., Dial, G.D. (1992). Changes in luteinizing hormone concentrations after abortion, parturition and weaning in primiparous sows. Anim. Reprod. Sci. 27: 169-182.

Stevenson, J.S, Cox, N.M., Britt, J.H. (1981). Role of the ovary in controlling luteinizing hormone, follicle stimulating hormone and prolactin secretion during and after lactation in pigs. Biol. of Reprod., 24:341-353

Tokach, M.D., Pettigrew, J.E., Dial, G.D., Wheaton, J.E., Crooker, B.A., Johnston, L.J. (1992). Characterization of LH secretion in the primiparous lactating sow:relationship to blood metabolites and return to estrus interval. J. Anim. Sci. 70:2195-2201.

Varley, M.A., Foxcroft, G.R. (1990). Endocrinology of lactation. J. Reprod. Fertil. Suppl., 40:47-61.

Wardlaw, S.L., Thoron, L., Franz, A.G. (1982). Effect of sex steroid on brain β-endorphin. Brain Res., 245:327-331.

Whisnant, C.S., Goodman, R.L. (1988). Effects of an opioid antagonist on pulsatile luteinizing hormone secretion in the ewe vary with changes in steroid negative feed-back. Biol. Reprod., 39:1032-1038.

Whisnant, C.S., Kiser, R.E., Thompson, F.N. and Barb, C.R. (1986). Opioid inhibition of LH secretion during the postpartum period in suckled beef cows. J. Anim. Sci., 63:1445-1448.